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The Development of Symbranchus marmoratus.

By

Monica Taylor, S.N.D., B.Sc.

With Plates 1—4 and 4 Text-figs.

Contents.

I. Introduction.

The Symbranchii are a small group of eel-like fishes which are generally placed near the Apodes in recent systems of classification, though lack of sufficient anatomical description and embryological material has made it difficult to determine exactly their affinities. Tate Regan, in his "Anatomy and Classification of the Symbranchoid Eels" (**9**), derives them from the Acanthopterous physoclists. The embryology of a Symbranchid has, up till now, not been investigated.

The following account deals with the South American species, Symbranchus marmoratus, which long ago

excited attention because, while possessing well-developed gill-
lamellæ on each of the four gill-arches, and typical Teleostean
aortic arches, it yet spent a considerable part of the year
buried in the mud like a Lepidosiren (5). Probably correlated
with this habit of burrowing, and with modifications in the
respiratory processes during the dry season, is the single
median ventral branchial opening—a character which is
described in the name Symbranchus. Other adaptations
to this burrowing habit are probably the great length of the
gill-chamber—the branchial opening being situated com-
paratively far back—the vascular character of its lining, and
also its almost complete separation from the exterior. Several
members of this group possess accessory respiratory sacs, and
J. Müller, in his work on the Myxinoids, says: " Es wird
wohl das obere Ende des Zungenbeins gemeint sein, denn
hier sehe ich bei Symbranchus eine blinde Vertiefung jedoch
ohne Sack, zwischen dem Zungenbein und dem obern Ende
des ersten Kiemenbogens."

I have found the diverticulum referred to by Müller in the
three adult specimens which I have examined—and indication
of its future position in the older larvæ. An account of its
structure will be given in the section on the alimentary canal.

The material for this work was obtained during an expedi-
tion to the Gran Chaco in 1907, when Dr. Agar made the
interesting discovery that the larva of Symbranchus has
very well developed pectoral fins. Hitherto the absence of
pectoral fins has been regarded as one of the characteristics
of the group. The material consists of eggs and larvæ
preserved immediately after they were taken from the nest,
and also of specimens reared under artificial conditions in
tanks and dishes of water. In the latter case it was possible
to record the rate of growth after hatching.

Dr. Agar has given an account of his expedition in his paper
on "Spermatogenesis of Lepidosiren paradoxa" (1), and
to his field notes I am indebted for all the details relating
to the nest of Symbranchus, the collection of the eggs, the
habits of the living creature, while what I say regarding

"movement of the fins" is taken verbatim from his notes.
I should like to take this opportunity of thanking Dr. Agar
for placing this unique material unreservedly at my disposal,
and for the kind interest he has shown in the progress of the
research.

My best thanks are offered to Professor Graham Kerr, in
whose laboratory much of the work was done, and who has
helped me throughout with constant advice and criticism.

I am greatly indebted to S^r· Veronica, S.N.D., for her
artistic illustrations.

Symbranchus, like many fishes and amphibians, exhibits
the phenomenon of colour-change in response to alteration in
the conditions of light and darkness, specimens fixed at
night being lighter in colour, because of the contraction of
the chromatophores, than those fixed in daylight.   The living
larvæ actively burrow among the weeds at the bottom of the
rearing tanks when the lid is removed—as though to escape
from the light.

About a dozen nests in all were found, the adult male, as in
the case of Lepidosiren, being present to guard the contents.
In many other respects the nesting habits of Symbranchus
resemble those of Lepidosiren.

The water in the swamps where the eggs were found was
commonly from one to four feet deep.   The mouth of the nest
opens on the bottom of the swamp and leads into a tunnel
running obliquely downwards into the mud.   At the end the
tunnel takes a more or less horizontal direction, and it is in
this part that the eggs are laid, there being little or no weed.

The eggs are translucent, and of a greyish colour.   It is
quite impossible to assign a definite age to the contents of
any one nest, as the eggs may show every variety in state of
development from "early segmentation" to "moving
embryos."   Neither is it possible to give any definite idea of
the rate of development before hatching.   However, in a nest
discovered on December 12th the eggs were of fairly uniform
and of early stage (Stage 5).   Some of these hatched out on
the 19th, the resulting larvæ being of the stage described as

24. Some of these same eggs, extracted and preserved on the 17th, are described as Stage 23.

For the following descriptions, whole and sectioned eggs and embryos fixed in corrosive acetic and formalin were examined, as well as stained and cleared preparations of blastoderms and of whole embryos removed from the underlying yolk mass. The external appearance of the embryo varies a little with the fixative employed, as may be seen by comparing Pl. 1, figs. 5 and 5 A. These two eggs are of about the same age, but that figured in 5 A was fixed in corrosive acetic, that figured in 5 in formalin.

For stages beyond 24 whole specimens stained and cleared in xylol were consulted as well as opaque specimens and sections.

II. General Sketch of Development.

The preserved specimens up to Stage 20 were mostly collected from three nests, and were fixed in formalin, warmed or cold. No eggs were found in the cœlom of female Symbranchus.

The laid egg is spherical and has a diameter of 3·4 to 3·5 mm. Its capsule is firm and somewhat tough and separated from it by fluid. A mass of what looks like a coagulated albuminous substance is present at the vegetative pole. When the capsule is removed this substance separates away, so that the egg appears to be sometimes no longer spherical but somewhat oblate, the equatorial diameter being the greater. It is also very brittle.

The youngest specimen in the collection is described as Stage 5 (Pl. 1, fig. 1). At this stage the blastoderm is very well marked. It looks like a white circular pad " let into " the yolk-mass, this pad being composed of small white cells and measuring about 1 mm. in diameter. It stands above the surrounding yolk to a height of nearly ·5 mm. The animal pole is flat in comparison with the distinctly spherically curved vegetative pole.

Celloidin sections through eggs of this stage show that this pad is more or less bi-convex in shape—9 to 10 cells deep in the centre. Already an outer layer of cells is becoming marked off from the general mass, i. e. an ectoderm differentiating. Three distinct zones can be seen in the subjacent yolk, from which the pad can easily be separated. Under the central deepest part of the blastoderm the yolk is finely divided, while the lateral parts of the " pad " overlie a mass of protoplasm studded with large nuclei. Here the outer rim of the blastoderm shows more obvious continuity with the rest of the egg than it does over the central portions. This protoplasmic mass no doubt functions in connection with the passing on of food material to the quickly dividing cells in the blastodermic area.

Protoplasmic masses and disintegrated yolk pass more or less suddenly into the large structureless yolk-blocks which constitute the vegetative part of the egg. A thin film of protoplasm acts as a covering to the yolk-mass, which is not yet covered by blastoderm. This covering is, however, a very slender protection, especially at the vegetative pole, where masses of yolk break away very readily when once the capsule has been removed.

Stage 7.—As the blastoderm grows the central portion appears to thin out, while the rim is thickened and less white in appearance. The yolk nearest the blastoderm is much lighter in colour than the rest, which is brownish-yellow— this difference in colour being the external expression of the yolk differentiations described above. The celloidin sections show an ectoderm, now two-layered, and an irregular and very small segmentation cavity.

Stage 10.—When the blastoderm has covered up about 100° to 120° the thickening of the rim becomes accentuated at one point to form the embryonic rudiment (Pl. 1, fig. 2, and Text-fig. 1). In the paraffin sections (Pl. 3, fig. 17) there is a space underlying the embryo rudiment which may be artificial or which may represent remnants of an archenteric cavity. Celloidin sections (Pl. 3, fig. 18) do not show any such

space, but the series is a slightly older one than the paraffin series. In the embryonic rudiment, as seen in a celloidin section, the thickened neural plate covered by ectoderm lies over an ill-defined endoderm, the yolk syncytium separating the latter from the yolk. The rest of the blastoderm is now two cells deep except at its advancing edge, where it is still thick.

TEXT-FIG. 1.
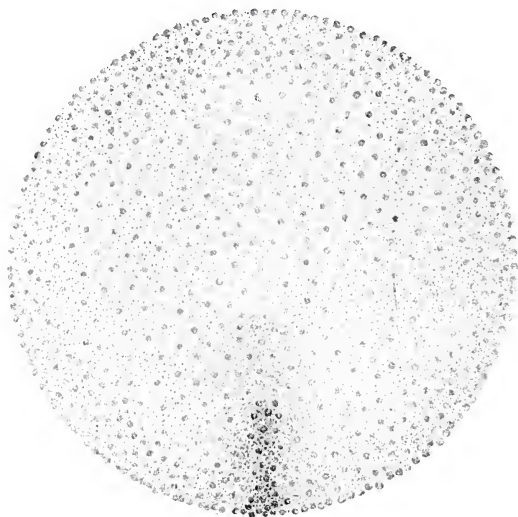
A microscopic preparation of the blastoderm of an egg of Stage 10, removed from the underlying yolk, stained, cleared and mounted in balsam.

Stage 11 is illustrated by Plate 1, fig. 3. The blastoderm is gradually extending over the egg, becoming much thinner as it does so, as though the increase in surface area were at the expense of depth. The embryonic rudiment is elongated to form a white streak, which is broad at the anterior end, narrower in the middle, and broader again where it gradually fades into the blastodermic rim. The elements forming this streak appear, in sections, to be very much pressed down by the outer ectoderm.

The small size of the egg and the corresponding small size and flattening of the embryonic rudiment make it practically impossible at this stage to do more than distinguish the brain rudiment from that of the neural tube and myotomes. A faint median longitudinal groove can be seen externally. A microscopic examination of sections of the egg shows that the broad anterior part of the rudiment consists of a wide-spreading, solid medullary plate overlying a one-cell-deep plate of endoderm, there being little mesoderm. In the middle parts of the rudiment a solid keel—the future spinal cord—is quite easily distinguishable from the mesoderm and from the endoderm. There is no certain trace of noto-chord yet.

The groove alluded to above is the external expression of a very shallow mid-dorsal depression found in the anterior parts of the embryonic rudiment, which disappears when the flat, wide-spreading medullary plate is converted into the solid uprising structure which forms the central nervous system just before the cavities are hollowed out.

Stage 17.—When the still uncovered circular yolk-patch measures about 48° the embryo rudiment is about 1·8 to 2 mm. in length (Pl. 1, fig. 4), though there are great variations, length being not always proportional either to differentiation of embryo or to size of uncovered yolk-patch. By this time the front part of the medullary plate has begun to rise from the egg, and to form the solid brain, and this uprising gradually extends backwards to the rest of the neural rudiment. The myotome rudiments are not yet differentiated into dorsal and ventral portions. The notochord is present, but its deriva-tion is difficult to observe. The solid rudiments of nose, eyes and ears are not yet distinct, though in the next stage—when the lumen of the nervous system appears—they are easily distinguishable in sections and their ectodermal connections can also be seen. Externally, however, these organs are not yet visible.

As development proceeds, liquefied yolk or oil, of which there is much in the yolk, seems to concentrate in front of

the embryo, and then to spread out somewhat so as eventually to extend over about half the sphere. This gives the egg a very characteristic appearance, the liquid yolk being silvery and glistening.

Stage 19.—By the time the embryo is 2 mm. or a little more in length the yolk has been quite covered in. The brain is hollow. The rudiments of eyes and ears are assuming a cavity ; the position of the principal cranial nerves can be determined, as well as that of mandibular and hyoid arches and the rudiments of the four branchial arches. A most careful search, however, has revealed no trace on the external surface of gill-slits, either in this or in later stages, the slits not becoming perforate till after they are covered over by the operculum, so that in this respect Symbranchus differs from other Teleosteans. The developing pectoral fins are conspicuous in sections at this stage. They contain the solid ventral muscle processes of three myotomes of the front part of the trunk. The innervation and muscularisation of the pectoral fins has been worked out by a study of the composition of the brachial plexus, an account of which will be given later on.

No trace of Kupffer's vesicle can be found in this or in earlier stages.

Stage 21.—The embryonic rudiment has now a ridge-like appearance. It curves round the yolk-sac, which has a diameter of 2·9 to 3 mm., through an angle of 170°. By the aid of sections the brain is seen to be divided into three parts, though the mesencephalon is not nearly so well marked off from the prosencephalon, as it is from the rhombencephalon. The optic vesicles are conspicuous, while the mandibular, hyoid and branchial arches with their nerve supply are to be seen as an unsegmented, translucent, tripartite mass lying on either side of the brain (Pl. 1, fig. 5). The fins look like two patches on the surface of the yolk, the ventral processes of the myotomes (Pl. 1, fig. 5A) like oblong blocks spread out on the dorsal part of the yolk-sac. The pericardial cavity is beginning to form. Pl. 1, fig. 5 gives

a view of an egg slightly older than the one just referred to. The main mass of the pectoral fins is about ·3 mm. from the embryo proper, so that the fins look like two white mammillæ apparently unconnected with it (see also Pl. 1, fig. 6, a slightly older stage).

Stage 23.—When the embryo is a little over five days old there is a curious proboscis-like structure (see Pl. 1, fig. 7), projecting freely from the extreme end of the creature more or less on a line with the mesencephalon. This rostrum is transparent and bluntly pointed and filled apparently with a clear fluid. The tail is curled, though still in contact with the yolk. The pectoral fins are about ·5 mm. in breadth and extend upwards and backwards over the yolk. The cartilaginous skeleton of the fin has made its appearance and is visible externally.

Stage 24 (Pl. 1, fig. 8) is the stage at which the embryo probably hatches out. The body of the creature has become much more cut off from the yolk, the rostrum projecting forwards anteriorly, the hind end being quite free. The heart is visible in the clear space separating the head from the yolk mass. The visceral arches are more conspicuous; eyes and nostrils are ventral in position. Two rounded optic lobes anteriorly and a well-marked very wide rhombencephalon posteriorly seem to be covered with a transparent lymph space continued into the rostrum already alluded to. The unpaired fin is beginning to grow.

Stage 25 (slightly more advanced than Pl. 1, fig. 8).— The embryo has now considerably lengthened, the posterior part being coiled as a result of this growth. As in the last stage, the head does not project freely from the yolk-sac except the rostrum, which is now extremely conspicuous, being about ·7 mm. long. In some of the specimens particles of mud and débris are attached to the rostrum at its tip, which suggested that this structure might perhaps function as a cement organ. A microscopic investigation of such specimens has, however, given little, if any, support to such an interpretation of the function of this curious appendage.

The organ seems to be a prolongation of the transparent tissues surrounding the dorsal part of the brain, filled with a lymph-like fluid without any apparent cellular elements and walled in by two layers of epidermis, the outer of which is flattened and horny (Pl. 1, fig. 11). In the specimens with the débris there is a slight thickening of the lower epidermis · cells in the neighbourhood of the débris which may possibly represent a vestigial cement-gland, but it has not been found possible to demonstrate a break in the continuity of the outer horny layer, which fact tells against such an interpretation of the organ. The line of demarcation between the mesenchyme of the head and the fluid contents of the rostrum is very definite.

There is a resemblance between the figures of the early developmental stages of the rostrum of Callorhynchus (10) and that of Symbranchus, and also a certain amount of resemblance between their structure in the early stages, for Schauinsland says that the rudiment of the rostrum of Callorhynchus is a large thin-walled sac covered with ectoderm and later becoming filled with mesoderm—a description which might apply in part to the organ in Symbranchus.

Large unicellular glands are very conspicuous objects in the ectoderm in sections stained in Heidenhain and eosin. They are most abundant in the epidermis of the fins, but occur also in the general epidermis, and are to be found, though sparsely, on the rostrum. These glands are evidently larval structures, for they gradually disappear as the adult stage is reached.

By this stage the dorsal and ventral median fins are well established and there is a cloacal opening which appeared in Stage 24, so that it is now possible to distinguish the anterior limit of the true tail. In dorsal view the only parts of the brain visible are the optic lobes and rhombencephalon because of the cranial flexure. In side view the infundibulum and hypophysis are conspicuous; olfactory organs and eyes have become more lateral in position. The heart is more clearly seen, the ear is still visible (in the xylol specimens) anterior

and dorsal to the branchial regions. An operculum barely covering the first branchial arch is visible in horizontal sections. The pectoral fin, the anterior edge of which curves backwards, now measures about 1·1 mm. in length. The liver is visible on the left side in the xylol specimens.

Dr. Agar describes in his notes a pigment-spot on the left side of the creature as being conspicuous in the fresh specimens. In the early stages, i.e. forty hours after hatching, this pigment-spot lies anterior to the middle point of the attachment of the yolk sac. Its position is relatively further back in the later stages of development and more superficial. No trace of this pigment-spot can be found in the preserved specimens, but in those stained and cleared in xylol the gall-bladder is distinctly visible in the position described in the notes, and a consultation of the sections leaves little doubt that the gall-bladder is responsible for the spot.

Stage 26 (Pl. 1, fig. 9).—The coiled portion of the body alluded to above straightens out as the creature grows, so that at this stage (26) a straight line joining the tip of the rostrum to the tip of the tail measures 7 mm. The rostrum has lengthened and lies parallel with the curve of the yolk-sac. The pectoral fins are about 1·5 mm. in length. The cerebellum rudiment is conspicuous (c. r., Pl. 1, fig. 9) ; the eye is more lateral in position. The operculum covers the first two branchial arches.

Stage 27.—Seventy hours after hatching the creature is about 9 mm. long, the true tail being about 2·5 mm. Its general appearance has, however, undergone little change from the conditions shown in Pl. 1, fig. 9. The pericardial space has increased in depth. The head is becoming more opaque.

In Stage 28, the rostrum, on account of the growth of the brain, nose and eyes would now be described more correctly as a dorsal cephalic process than as an anterior one. The subintestinal vein (Pl. 1, fig. 9) is conspicuous in the figures of this and succeeding stages, and a sketch of the living creature among Dr. Agar's field notes shows that it is

evidently a striking object in the living larva. The sections
show that the blood-vessels of the fins are well established,
the arterial supply being derived from the dorsal aorta in the
neighbourhood of the pronephros immediately behind the
heart, the venous blood being returned to the duct of Cuvier.

In Symbranchus it is impossible to say dogmatically that
the artery to the pectoral fin has been derived from an inter-
segmental artery (i. e. is a primary subclavian artery[1]) because
of the precocious development of the fin in question, but a
comparison of the early development of the fin artery with
the intersegmental arteries (Pl. 4, fig. 27) that arise later on
when the myotomes are fully formed shows that the arterial
supply to the fins is intersegmental in nature. Three
myotomes take part in the formation of the fin; whether the
subclavian in Symbranchus represents the intersegmental
artery of one or the fused intersegmental arteries of all three
myotomes it is not possible to say.

Stage 29.—There is a shrinking in the rostrum at this
stage. From now onwards this structure gradually diminishes
in size. Chromatophores appear, being confined to the head
at first. On the right-hand side of the creature the cœlom is
visible externally, the liver being conspicuous on the left.

In Stage 29 the inclusion of the yolk in the abdominal
cavity commences. This has an important effect on the
contour of the creature, as may be seen by comparing Pl. 1,
fig. 9, and Pl. 2, fig. 13.

Stage 30,[2] (Pl. 1, fig. 10).—The creature at this stage is
13 or 14 mm. in length, its rostrum being 1 mm. long. There
has been a great forward growth of head, which is assuming
a definitive form, the optic lobes are covered with a pigmented
epithelium, cranial flexure has almost disappeared. The eyes
are fully developed, and in contrast to later stages are only
just visible in dorsal view.

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[1] Hochstetter in Hertwig, O., ' Handbuch der vergleichenden und
experimentellen Entwickelungslehre der Wirbeltiere.'

[2] In writing the description of this stage I have been aided by a large
drawing made by Dr. Agar from the living larva.

The living larva makes frequent swallowing movements and the gill-lamellæ are differentiating. Thus preparations for branchial respiration are afoot. Hitherto respiration has been effected mainly by the pectoral fins. The larva has also begun to feed, its food under artificial conditions consisting of earthworms and liver. In the living specimen dorsal aorta, posterior cardinal, caudal, subintestinal veins are well marked, while the ventral aorta and the four aortic arches can be seen. The urinary bladder, the widened out posterior parts of the fused archinephric ducts, first visible in Stage 28, is conspicuous in the xylol specimens of this stage. It now measures 1 mm. in length and lies dorsal to the rectum, opening to the exterior immediately posterior to the anus, the cloaca having flattened out.

Description of the Pectoral Fins.—It has been shown that in the early stages, 18 to 21, the pectoral fins consist of mesoderm masses—the ventral processes of certain anterior myotomes—covered by a two-layered epidermis. Very quickly, however, these masses begin to differentiate. Cartilage derived from the cells forming the central part of the fin-rudiment appears at Stage 24 to form the skeleton, which is visible externally, and the muscle-fibres are differentiated. The main mass of the fins, however, is mesenchymatous, the increase in size being mainly due to increase of this tissue. The fins are very transparent and beautiful objects as well as conspicuous by their large size, the longest diameter at Stage 30 being 2·5 mm. When living they are quite red because of the rich network of capillaries. They are roughly semicircular in shape, the diameter of the circle lying nearest to the yolk-sac. The cartilaginous part of the fin is triangular (Pl. 1, fig. 10 and Pl. 2, fig 12), the apex of the triangle consisting of a rounded knob, to which most of the muscles are attached. This rounded knob fits into the shoulder girdle, dividing the latter into a dorso-lateral and ventro-lateral portion. The shoulder-girdle is little more than a thin lamella of bone surrounded by much muscle. The cartilage

in the fin is differentiated earlier than the bone of the shoulder girdle. I have not observed any trace of cartilage in the development of the girdle.

The unicellular glands already described are visible externally under a lens.

Three main blood-vessels are distinctly visible in the fin, one afferent supplied from the dorsal aorta which runs into the fin dorsal to the "skeleton," and which breaks up into capillaries, and two efferent. These efferent vessels make their appearance in the circumferential regions of the fin. One of them then takes a route dorsal to the "skeleton," the other ventral. Both pass into the body proper anterior to the nodular end of the cartilaginous skeleton and to the shoulder girdle, and having joined, they pass backwards parallel to the inferior jugulars to open into the heart at a little distance behind the junction of the duct of Cuvier with the heart. This backward course is due to the fact that the position of the heart has been changed relative to the fins since the veins were first formed.

The innervation and muscularisation of the fin has been worked out by reconstructions made by the Graham Kerr method (4) from sagittal sections. In the earlier stages transverse sections are useful. The occipital arch was taken as a fixed point, myotomes and nerves behind this arch being numbered 1, 2, 3, etc. It can be shown that spinal nerves 1, 2 and 3 innervate the fin. This is an indirect method of proving that it was the ventral process of the first three trunk myotomes that were concerned in the formation of the fin, but as late as Stage 26 there is no brachial plexus — each nerve enters the fin quite independent.

In Stage 28 the ventral ganglia of the first and second spinal nerves, which, unlike the other spinal nerves, were not separated by neural arches, are fusing one with the other. This fusion, which is probably correlated with the lengthening-out process of the branchial cavity already alluded to, has gone still further in Stage 29, though the double character of the apparently first spinal nerve is still evident. The brachial

plexus begins to differentiate in Stage 28, the plexus being somewhat diffuse and wide spreading at first.

A reconstruction of Stage 30 shows that the fused spinal ganglia of nerves 1 and 2 forms a large structure just outside the occipital arch. It also shows that the shoulder girdle has suffered a backward migration relative to the spinal nerves, for nerves 1 and 2, now fused into one nerve, and nerve 3, to a less extent, have to run backwards more or less parallel to the vertebral column to join the brachial plexus. This backward displacement of the shoulder girdle has resulted in Stage 33 in the further elongation of the nerves running to the brachial plexus. A small branch of spinal nerve 4 joins the plexus, though I have no definite developmental evidence that myotome 4 has played any part in muscularising the fin.

Movements of the Fins.—As soon as they are hatched the larvæ begin to make constant movements with their great vascular pectoral fins, doubtless for respiratory purposes; the motion is jerky and very characteristic. Each fin is moved through an angle of about 180°, being flat against the body at the end both of its forward and backward movement, and the direction is generally opposite on the two sides, one fin being flapped forward while the other is being turned back. This produces an impression on the observer watching the larvæ from above as if the two fins were a single bar pivoted through its centre to the body of the larvæ and surging backwards and forwards in a jerky manner. This form of motion is not absolutely fixed, however, and when the larvæ were disturbed they were sometimes seen to flex and extend their fins together, thus using them as ordinary locomotor organs.

The further history of the pectoral fins is given under Stages 32, etc. No trace of pelvic fins has yet been forthcoming though a careful search has been made.

A fuller history of the gill-chamber and the opening is given in connection with the development of the alimentary canal; here these structures will be only briefly described. Gill-slits such as are seen, for example, in a developing trout

are never visible on the exterior of Symbranchus. The branchial chamber, which is gradually hollowed out as development proceeds, develops a connection with the exterior by means of a small pore on each side, microscopic in character, dorso-lateral in position. These openings lie behind the posterior edge of the operculum and are found in the early stages (up to Stage 29), when the pericardium is still ventral to the pharynx. At Stage 30 the heart has suffered a backward migration relative to the gill-chamber, and by this time an additional opening to the exterior from the latter has been burrowed out on the ventral side of the creature. This ventral opening becomes continuous with the dorso-lateral pores on each side, thus forming a single crescent-shaped opercular opening, the horns of the crescent being posterior and dorso-lateral in position. If the ventral part of this opening could be obliterated then the opercular conditions of Symbranchus would resemble those of an ordinary Teleostean, i.e. there would be two lateral openings. However, it will be shown that, as development proceeds, the operculum continues to grow backwards, and as it does so it fuses with the body-wall, so that eventually a single median ventral branchial opening is the result. Co-extensive with this backward growth of the operculum is the lengthening of the branchial arches and of the whole gill-chamber.

Stage 32 (Pl. 2, fig. 13).—About eight days after hatching the creature begins to assume something of its adult appearance and to use its gills quite freely. It is about 18 mm. long, having a tail of 5 mm. in length. The yolk-sac has almost lost its globular appearance, and the inclusion of the yolk in the abdominal cavity, already alluded to, has continued. The yolk enclosed in the body of the creature now extends to within 1 mm. of the anus. The body anterior to the heart is much elongated, the most anterior point being at a distance of 2·5 mm. from the yolk-mass. The pectoral fins have, however, not grown in proportion to the rest of the animal: they are about the same actual size as in Stage 30. Hence, on account of the general growth of the creature,

they are not so strikingly conspicuous as in the younger stages.

The increase of distance between the anterior point and yolk-mass is due not only to the lengthening of the gill-chamber, but also to the formation of a snout, which is now quite clearly defined, and which has grown out from under the cerebral part of the brain. The telencephalon is in consequence now nearly at the same level as the optic lobes. This snout formation has the effect of carrying the eyes to a more anterior position: they now lie under the cerebral regions quite anterior to the optic lobes. The plane of the mouth opening becomes rotated upwards so as to assume a definitive horizontal position.

The rostrum has almost disappeared. Chromatophores are increasing in number; the creature is more opaque, so that liver and bladder are less conspicuous in the xylol specimens. The median dorsal fin in Stage 32 is dying away anteriorly. It begins to rise from the general surface behind the gill-chamber.

Stage 33 (Pl. 2, fig. 15).—Thirteen days after hatching the creature is about 21 mm. long and the yolk-sac has completely disappeared. A little mound—striking, because of the absence of pigment, which by now is abundant all round it—is all that remains of the rostrum. The root of the pectoral fins has become shifted ventrally nearly to the mid-ventral line. The lateral parts of the crescentic opercular opening of earlier stages are gradually being closed up, so that in this stage the opercular opening is quite ventral though relatively larger than in the fully adult condition.

The posterior parts of the outer wall of the gill-chamber, especially in the neighbourhood of the opening, are exceedingly thin. Normally the exit from the gill-chamber is closed and concealed, except when water is being actually expelled from it. The shoulder girdle and the attachment of the fins are now quite anterior in position to the hind end of the branchial chamber, the opercular flaps overlapping the base of the fins, which are now somewhat crumpled instead of

being flat. It will be remembered that in Stage 30 branchial respiration was just beginning, and that the pectoral fins ceased growing as soon as branchial respiration was well established. In Stage 34 the fins shrivel and fall off bodily, the discarded fins measuring about 1·5 mm. in length. The "stumps" are, however, recognisable in the sections for some time longer. Quantities of these discarded fins are to be found lying on the bottom of the tanks containing living larvæ of this and the succeeding stages. The fins in Stage 30 are situated just posterior to the opercular opening. In Stage 32, however, the hind end of the operculum has reached the point of origin of the fins, as may be seen in Pl. 3, fig. 19. In Stage 33 operculum and branchial cavity have become elongated to such an extent that the fin "stalks" are actually covered by the operculum, and can only gain exit from the branchial chamber on the ventral side (Pl. 2, figs. 14 and 15), the gill-chamber opening by this time having nearly assumed its adult position and size.

The pressure of the edge of the operculum on the base of the fin is possibly responsible for the degeneration of the tissues in the "stalk"—thus enabling the main part of the fin to drop off. On the other hand, it is quite possible that the "shedding of the fins" is a natural selection phenomenon, the fins tending to degenerate at their base at the period when they become covered by the operculum without there being any direct mechanical effect. The next stage (34, which is not figured) is marked by absence of fins, a more anterior position of the eyes, and by the extension of the liver to the level of the anus.

In Stage 35 (Pl. 2, fig. 16) the larva has practically assumed its adult form, though head and branchial regions are perhaps more distinctly distinguishable from the rest of the body than they are in the full grown specimen. There is no sign of pectoral fins and no trace of rostrum. The crea-ture is eel-like in shape, the body behind the opercular cavity being almost perfectly cylindrical, while the hinder part of the trunk, becoming gradually more laterally com-

pressed, merges eventually into the tail.   The ventral ends of the myotomes have now met ventrally and form a complete investment of muscles to the trunk.   Chromatophores are still confined to the dorsal and lateral parts, the ventral surface being quite colourless.

The length is 26 mm.   The true tail is distinguished by the continuous median fin, which gradually rises from the dorsal surface in front of the anus, is continued round the tip of the tail, and dies away on the ventral side at a point midway between the extreme hind end and the anus.

Although this continuous fin is only conspicuous in the tail, there is, however, a low-lying dorsal fin-like structure extending as far forwards as the pericardium.   A similar structure continues forwards—the ventral portion of the fin—this being the last remains of the transparent tissues traversed by the sub-intestinal vein of the earlier stages.

The tail fin is still transparent, though branching chromatophores are now more copiously present.   The anus is about 9 mm. from the tip of the tail.

The extreme front end of the snout is squarish, the upper jaw longer than the lower, so that the mouth is slightly ventral.   The gape is not great, and is well protected by the thick fleshy upper and lower lips.   Just inside the mouth-cavity on its dorsal surface there are some forwardly directed blind pockets, the floors of which form valvular flaps for the more efficient closing of the mouth aperture.   Both anterior and posterior nares are visible, the latter, which first appear in Stage 30, being situated just in front of the eyes, the former at the very tip of the snout on either side of the middle line.   The posterior nares do not arise by a division into two of one single olfactory opening, but by a hollowing out of a channel to the exterior.

Sensory canals, which first make their appearance in Stage 34 just posterior to the olfactory organ, are present in the head, opening to the exterior at intervals of about ·08 mm. The epithelium of the snout is richly glandular, as, indeed, is the epithelium of the trunk.

The opercular cavity stretches backwards to a distance of 4 mm., the opercular opening being now quite the small median ventral aperture so typical of the adult.

The ventral aorta is very long, the distance between the heart and the fourth aortic arch being 1·6 mm. In the full-grown specimen the distance between the tip of the snout and the pericardium is roughly a quarter of the whole length, and as a rule the pericardium bulges out slightly and is consequently visible exteriorly. The eyes are smaller in proportion than they were in the earlier stages.

III. Notes on the Development of Certain Systems of Organs.

a. Alimentary Canal and Certain of its Glands.

As has been shown in the preceding account, gastrulation occurs at about Stage 8. Celloidin sections through the egg of Symbranchus at Stage 10 and onwards show that yolk endoderm (yolk syncytium) underlies the whole of the blastoderm. In the embryonic rudiment (Stage 10) endoderm cells can be clearly distinguished from the overlying thickened neural plate (Pl. 3, fig 18, *n.p.*). The nuclei of this endoderm, which does not extend beyond the limits of the rudiment, in contradistinction to the large nuclei of the yolk endoderm, are small and oval-shaped, the long axis of each being usually at right angles to the axis of the embryonic rudiment. The line of demarcation between the endoderm of the embryonic rudiment and the yolk-syncytium is well defined in both paraffin and celloidin sections.

As the development proceeds, mesoderm gradually separates apart the endoderm and the overlying neural rudiment, and the notochord is formed. I have not been able to observe the process by which the notochord is derived from the endoderm, but by Stage 16 this structure is easily recognisable as a solid cord of cells.

In Stage 18 the stomodæum begins to grow in from the deep layer of ectoderm. It is very difficult to fix precisely

its internal limits, but the balance of evidence from many sections seems to show that it does not extend inwards beyond the infundibulum and the hypophysis. The outermost horny layer is not involved in the growth in any way. The stomodæum is, of course, quite solid. The flat, laterally spreading endoderm of the preceding stages is becoming a more or less rounded, solid mass of cells in the stomach rudiment, which is very closely apposed to the overlying notochord.

The pharyngeal rudiment overlies the pericardium in Stage 19. The solid endodermal cleft rudiments are not yet clearly distinguishable.

Transverse sections of embryos of Stage 21 show that the anterior parts of the alimentary canal, including the visceral clefts, are gradually moulded during the course of development out of a solid mass of tissue, swollen out laterally and tapering towards the central axis, which begins posterior to the infundibulum. The position of the solid hyobranchial cleft rudiment can now (Stage 21) be defined, while behind the branchial arches the rudiment of the œsophagus can be distinguished as a solid mass, broader than deep. Posteriorly, a solid cord of endoderm cells, the gut-rudiment, stretches with little change, save a gradually decreasing diameter to the hind end. The process of separation of this definitive endoderm from the syncytial yolk-endoderm has not been observed.

The alimentary canal is still solid at Stage 24, the cloacal opening being established by Stage 25. The lining of the stomodæum at Stage 24 is differentiating into a very regular epithelium. Mandibular and hyoid arches can clearly be distinguished in sagittal sections, while in the branchial rudiment, although the clefts have not yet been hollowed out, it is easy to see the number of arches, because of the epithelial lining of each.

Transverse sections at Stage 24 are particularly interesting because they show clearly an ingrowth of ectoderm in connection with each of the branchial cleft rudiments. The

branchial rudiment is still solid as explained above. These
ingrowths of ectoderm are quite masked externally by the
outer horny layer, which is not involved in the ingrowth. If
this deep-seated layer of ectoderm is a true ectoderm—and
there seems no reason to doubt it—then it seems pretty clear
that each of the branchial clefts has a lining of ectoderm.

The pharyngeal rudiment behind the fourth branchial pouch
tapers off to a solid cord of cells, the diameter of which
gradually diminishes. Since the pericardial rudiment only
extends to the commencement of the œsophagus, the ali-
mentary canal beyond this point, while having no organic
continuity with, yet lies directly over, the great yolk-mass.
The differentiating stomach, presumably from lack of space in
a ventral direction, expands laterally to the left. The dorsal
mesenteries are for the same reason not vertical and median,
but more or less horizontal. The liver appears at this stage
as a solid lateral (to the left) outgrowth of the alimentary canal.
On the right, dorso-lateral in position, is the dorsal pancreatic
rudiment, solid, surrounded by and passing without any sharp
boundary into the surrounding mesenchyme, which forms the
spleen rudiment. This latter arises in connection with venous
blood, which is part of the liver and yolk-sac circulation. It
will be seen in the section on the vascular system that the
subintestinal vein breaks up into capillaries on the yolk-sac,
and that as regards circulation the liver forms part of the yolk.
The spleen, then, has the usual teleostean connection with the
blood of the subintestinal vein.

In Stage 25 the operculum—a typical teleostean one—has
grown backwards to the level of the second gill-arch, and the
gill-cavity begins to differentiate. Chinks appear in the solid
cleft rudiments separating apart the flat epithelium lining of
the two sides of each slit. The arches are not at this stage
the long curved structures which they eventually become;
almost the whole of one arch is visible in one transverse
section. The branchial rudiment no longer projects to the
exterior as it did in earlier stages. A study of Pl. 3, fig. 20, a
horizontal section through an embryo of Stage 25, will show

that the part of the branchial rudiment not yet covered by the operculum is overlaid by the tissue masses of the body wall, what is equivalent to the posterior wall of the last cleft extending forwards so as to overlap the cleft rudiments and being continuous with the backwardly growing operculum. No space exists between the surrounding tissues and the three hinder arches, but between the backwardly growing operculum and the dorsal part of the first gill-arch a space is being hollowed out and lined by epithelium. The chinks in the solid cleft rudiments already described and this small space represent the opercular cavity at this stage. There is no communication between it and the exterior.

The liver shows signs of histological differentiation, its cells no longer resembling those of the alimentary canal, but being distinguishable from the latter by their paler staining properties. Blood-corpuscles are plentiful in the developing liver. The spleen is bulky.

Posteriorly the alimentary canal passes into a dilatation which receives the kidney ducts. This is the cloaca.

In Stage 26 hyomandibular and hyoid arches are more clearly visible in sagittal sections. The gill-arches have increased in length, and are, therefore, more curved; the operculum has grown further back. There are now two small openings behind its posterior edge, which is at about the level of the second gill-arch, dorso-lateral in position, leading into the small differentiating opercular cavity. They each represent a small dorsal portion of the lateral opercular opening such as is found in many teleosteans. Ventrally the cavity is not yet hollowed out. A transversely directed slit-like lumen commencing behind the level of the hyoid separates roof from floor in the developing pharynx. The œsophagus is solid, but the rest of the canal is gradually acquiring a lumen.

The dorsal pancreas more or less surrounds the alimentary canal and one lobe has grown tailwards for a distance of 180 μ (Pl. 3, fig. 21). The spleen has now attained to a diameter greater that that of the stomach. The bile-duct and gall-bladder rudiments differentiate at this stage out of the

solid stalk connecting liver and alimentary canal. The lumen of the bile-duct develops from the alimentary canal outwards.

In the next stage (27) the formation of the tongue has begun. Its adult relations are illustrated by Pl. 3, fig. 22, there being nothing of special interest in its development. The gill-cavity is still small though the branchial apparatus has elongated considerably. The thymus rudiment first occurs here. It develops on the outer dorsal side of the four branchial clefts, 2, 3, 4, and 5.

The ventral pancreatic rudiments are solid outgrowths from the bile-duct. They meet the dorsal pancreatic rudiment so that at its hinder end the stomach is almost surrounded by pancreas. The adult pancreas would seem to be mainly the derivative of these ventral rudiments. The gall-bladder, originally solid, is being hollowed out. The spleen is very conspicuous. The temporary cloaca has become quite flattened out.

Stage 29.—A median sagittal section at this stage shows, in the constriction marking the origin of the tongue, the rudiment of the thyroid which is a solid derivative of the floor of the pharynx. (In Pl. 3, fig. 22 this gland is seen at Stage 34.) The mouth is not yet open, but the rest of the canal has a lumen. The bulbus lies directly under the second gill-arch. In Stage 29 there is the first trace of respiratory tissue. The two pore-like openings into the gill-chamber are shown in Pl. 4, fig. 23—a transverse section through this stage. The pancreas is very much invaded by the mesenchymatous vascularised masses which constitute the spleen rudiment. The posterior part of the liver widens out so as to cover in the dorsal part of the yolk, both right and left.

The alimentary canal has almost reached its adult structure in Stage 30. The larva is now capable of feeding and makes frequent swallowing movements, the respiratory lamellæ on the gill-arches being well developed. The heart lies posterior to the gill-chamber now (compare Pl. 4, fig. 23), and an opening from the latter on the ventral and ventro-lateral surface of the creature has been burrowed out. This is con-

tinuous into the dorso-lateral pore-like openings of the preceding stages, and is the functioning "exit" from the gill-cavity already described in Part I (compare Pl. 3, fig. 19). Later on the lateral portions become obliterated and the single median ventral opening is the result.

The alimentary canal of Symbranchus is straight, running from mouth to anus without bend or curve, showing little change in diameter, and very little to distinguish externally the different parts, at any rate behind the pharynx. But on dissecting open the canal of the fully adult Symbranchus, the surface appearance of the interior shows quite clearly œsophageal, stomach and intestinal areas. The sections at Stage 30 and onwards show that the stomach is of slightly wider calibre than the œsophagus, and that the characteristic macroscopic appearance is due to the presence of glands. Externally and microscopically it is possible to distinguish cardiac from pyloric part of the stomach, the latter having stout muscular walls. A pyloric valve, which, however, only begins to differentiate in Stage 32, connects the pylorus with the duodenum. The inner lining of the œsophagus and stomach is thrown into longitudinal folds, in sharp contrast to the intestine, which is honeycombed.

The thymus at this stage is very conspicuous in both sagittal and transverse sections. The separate rudiments have all joined to form one large gland.

In a median sagittal section the thyroid is distinguishable as a rectangular band of cells, stretching backwards from the root of the tongue to the first gill-arch. Transverse sections show that anteriorly the thyroid bifurcates. The structure at this stage is not glandular. The liver is like that of the adult, and the gall-bladder is walled in by two layers of flat epithelium.

At Stage 32 the pyloric valve is differentiated. The pyloric part of the stomach projects back like a spout into the duodenum. Finger-like protuberances of the latter are packed all round the anterior part of this spout, thus hiding the constriction that would otherwise exist between the

pylorus and duodenum. The blind, pocket-like outpushings, mostly on the ventral side in the beginning, can easily be seen at this stage, growing forward more or less parallel with the pyloric part of the stomach, in the mesodermal covering of the canal. (In Pl. 4, fig. 24 a transverse section through the valve at Stage 35 is given.)

The special character of the pyloric valve just described and the study of its development suggests one or other of the following hypotheses :

(1) That these finger-like outpushings of the duodenum represent degenerating pyloric cæca, or—

(2) That pyloric cæca have been evolved by an elaboration of these cæcal outgrowths consequent upon their becoming free from the muscular coat of the alimentary canal.

An examination into the condition of pyloric cæca in Teleosteans seems to furnish evidence in favour of either interpretation. Many Teleosteans are without pyloric cæca, and the number of these cæca when present and their arrangement is extraordinarily varied.

Among Ganoids, Acipenser, the most primitive, is possessed of pyloric cæca. It is interesting to note that the early appearance of the cæcal rudiments, as described by A. Nicholas (7), recalls the adult condition of the pylorus arrangements in Symbranchus, only, that in the case of Acipenser, the outpushings of the duodenum, while directed towards the stomach, do not fuse with the walls of the latter —the muscular coat of the alimentary canal is involved in the outpushing.

In Amia the pyloric cæca are absent, but the pyloric valve, as described by Piper (8), resembles quite closely that of Symbranchus.

Among Teleosteans the pyloric valve of the eel, where pyloric cæca are absent, is of the Symbranchus type.

Pyloric cæca are absent in Cyprinoids, Labridæ, Gobiiæ, Blennidæ, Syngnathus and Cobitis—forms in which there is no obvious stomach. In Blennius pholis and Carassius auratus there is no specialisation of the pyloric valve, there

being just a simple ring-shaped opening, but there is a long, very much coiled intestine. If there is a similar absence of a specialised valve combined with the presence of a very long intestine in the other members of the above-mentioned orders, then it would seem as though the absence of pyloric cæca and any specialisation of the pyloric valve in these Teleosteans were compensated for by the presence of a very long intestine.

A comparison of such forms as Cottidæ with one of the Catosteomi (Stickleback) is instructive as illustrating how the degeneration of pyloric cæca (according to Goodrich (3) pyloric cæca are lost, as a rule, in this suborder) may possibly bring about an appearance such as that described in Symbranchus. In Cottus the short finger-like processes lying round the front part of the duodenum, with their blind ends lying on the stomach, form a circle of pyloric cæca. A dissection of this part of the alimentary canal reveals a rosette-like valve (the protrusion of the stomach into the duodenum) surrounded by the individual openings of the blind sacs.

At first sight there is no constriction visible between the stomach and intestine in the stickleback (Gasterosteus aculeatus), but the constriction is really present though hidden by two broad-mouthed blunt bulgings of the duodenum in the direction of the stomach. One of these pockets is bigger than the other as a rule (in one specimen examined the smaller of the two was very small). Both are well bound down to the alimentary canal by connective tissue— in fact, they seem to show a tendency to fuse with it. The valve is of the same type as that of Symbranchus, so that, should the pocket-like outpushings become fused with the alimentary canal, then the likeness between the pyloric arrangements of the stickleback and those of Symbranchus would be perfect.

In this case it would seem that, if the pyloric cæca are really disappearing in the Catosteomi, then the Symbranchus pyloric valve is the result of degeneration of pyloric cæca—an indication of specialisation in Symbranchus.

Against such a theory, however, it must be remembered that this form of valve occurs in Lung-fishes as well as in Amia, and that it is more primitive in nature than cæca, a quite similar valve developing in other cases (cf. various insects).

Stage 35, etc.—Little more remains to be said. The pyloric valve is fully developed in Stage 34. In Stage 36 the mouth is provided with unicellular glands and numerous papillæ. A rectum can also be distinguished, being of slightly wider diameter than the rest of the intestine and its commencement being marked by a sphincter muscle.

The yolk-mass gradually diminishes, being pushed into a dorso-lateral (right) position by the encroachment of the developing viscera, the subintestinal vein being, however, always in close connection with it. To the end of its existence it is always surrounded by a yolk-syncytium.

Thymus and thyroid increase in size. The former is a dense mass of deeply staining units interspersed with blood-corpuscles and is a conspicuous object both in transverse and sagittal sections. At the hind end of the thyroid a central lumen filled with a structureless colloidal substance is surrounded by cells. In the adult condition the liver is elongated and unilobed. Its anterior end stretches almost to the pericardium, and is on the left side of the alimentary canal, but together with the great length there is a slight twisting of the long lobe, so that for part of its course this lies ventral to the alimentary canal, while its hind part (which reaches almost to the anus) is on the right of the intestine.

The gall-bladder is large and conspicuous. It lies in the liver, its ventral surface being just flush with that of the liver.

The adult pancreas is a comparatively compact and very much elongated gland. It reaches anteriorly to the gall-bladder, circumscribes the alimentary canal at the pylorus, in which place it attains its greatest bulk, gradually dying out in the hinder intestinal regions. The anterior part is dorso-lateral, the posterior part beyond the pyloric valve ventro-

lateral, both anterior and posterior parts lie between the alimentary canal and the liver (Pl. 4, fig. 24).

The spleen is a compact, lenticular-shaped structure more posterior than the gall-bladder, lying near the liver to the right of the intestine.

Yolk and Alimentary Canal.

In Symbranchus, as elsewhere, in the early stages, centres of growth are characterised by the presence of yolk, which is being reduced to fine dust-like particles by the activity of the intermingled protoplasm. In Stages 18, 21 and 22 large quantities of such yolk in process of assimilation with large, deeply staining yolk-nuclei are to be found under the hind end of the embryo. As the embryo increases in size a strand of this yolk-syncytium, with its characteristic nuclei, extends into the free tail part of the embryo immediately over the differentiating subintestinal vein. Pl. 4, fig. 25 is a sagittal section through an embryo of Stage 24. A somewhat similar condition of things is described by Assheton (2) for Gymnarchus niloticus. Similarly in later stages an anterior plug is visible (Stages 29, 30 and 31) behind the pericardium. This plug (Pl. 4, fig. 26) in Stage 31 becomes more or less separated from the rest of the yolk, and has much the appearance of a group of round and highly reticulate large nucleated egg-cells. These cells lie in the mesenchyme immediately posterior to the pericardium and ventral to the hinder part of the œsophagus.

Pharyngeal Pouch.

The whole of the lining of the gill-chamber of Symbranchus as well as the roof and floor of the mouth is provided with a rich network of blood-vessels forming a respiratory tissue. Anterior to the first efferent branchial the aortic roots of each side give off a large artery which breaks up into innumerable capillaries in the roof of the mouth. This blood is drained back into the anterior cardinals.

Reference was made in the introduction to a "pouch" or "pocket" given off from the roof of the mouth. Traces only of this "pouch" can be found in the young creature, but sections through the adult show that it is an invagination of the lining of the gill-cavity, probably a device for increasing the area of the respiratory surface. This invagination begins immediately behind the attachment of the first branchial arch to the pharynx, and the pocket so formed extends backwards with a gradually decreasing diameter to the attachment of the fourth arch. The opening into the pocket is large, longer than broad.

(b) The Excretory System.

Solid pronephric nephrotomes are discernible about Stage 18.

There is little to distinguish the rudiment of the pronephros from that of its duct. The nephrotome of the third trunk myotome appears to give rise to the functional pronephros, those of succeeding segments forming the archinephric duct. It is not possible to say whether other segments besides the third have any share in the formation of the pronephros, but in the light of subsequent events it does not seem likely.

The separation, which begins at Stage 20, of the various nephrotomes from their respective segments to become converted by a process of fusion into an archinephric duct seems to take place practically simultaneously—the hinder parts of the duct being formed as soon as the anterior portions.

Stage 21.—The third nephrotome has become more deep-seated. Although still solid it has increased in size because of the development of lacunæ, these lacunæ being the first indication of the pronephric chamber. The archinephric duct rudiment is still solid, round and cord-like, lying approximately on a level with the dorsal aorta.

Stage 24.—The rounded pronephric chamber rudiment is now being converted into a typical Malpighian capsule, and the formation of a glomerulus from the dorsal aorta has

begun. In its development and in its fully formed condition this pronephric chamber exactly resembles the Malpighian capsules of the mesonephros, though the former is larger than the latter. The glomerular rudiment is formed of deep-staining cells interspersed with blood-corpuscles, the whole forming a little clump lying in the cavity of the pro-nephric chamber, the lining of which is exceedingly thin. This clump lies on that side of the chamber which is nearest to the dorsal aorta rudiment.

The cells of the archinephric duct rudiment are assuming an epithelial character, and in some places there is a lumen.

Both ducts have become more deep-seated and now lie ventral to the dorsal aorta. The inter-renal vein appears at this stage.

Stage 25.—The pronephros has increased greatly in size, and the two pronephric chambers with their respective glomeruli are very conspicuous structures in horizontal sections. They lie in contact with the paired aortic roots just as these are joining up to form the dorsal aorta. An opening from the short pronephric tubule into the archi-nephric duct is established, the duct being now perforate throughout its length.

Stage 26.—The pronephros has reached its maximum size. The glomerulus of each chamber is conspicuous, and the opening into the tubule is funnel-shaped. There is no obvious boundary between tubule and duct. Anteriorly the latter is becoming coiled, posteriorly, it is quite straight. Its opening into the temporary cloaca has already been mentioned in Part I.

In Stage 26 the pronephros has become more deep-seated, lying immediately ventral to the dorsal aorta, which at this point gives off a cœliaco-mesenteric artery behind the aortic roots. The archinephric duct is surrounded by blood, the posterior cardinals anteriorly, and the inter-renal vein pos-teriorly, bathing the ducts on their inner side, while on the outer side is blood which communicates with the segmented somatic veins. There is no sign of the " pseudo-lymphatic "

tissue except at the extreme anterior end of the archinephric duct, where a patch of deeply staining mesoderm may possibly represent this tissue.

Stage 27.—At this stage the glomeruli are supplied from the cœliaco-mesenteric artery, the pronephros being quite ventral to, and much deeper than, the dorsal aorta. The " pseudo-lymphatic " tissue has increased, and is no longer confined to the anterior end of the kidney rudiment. The tissue at this stage consists of scattered masses, deeply stained, lying in the neighbourhood of the ducts and blood-vessels.

Stage 28.—The archinephric duct is more coiled and the pronephros has become more posterior relative to the pectoral fins. The urinary bladder has increased in length.

Stage 30.—At this stage the larva is capable of feeding and is breathing by means of its gills. Since the first rudiments of the mesonephros are barely distinguishable in Stage 29, it seems probable that the pronephros is the functional kidney at this time. The " pseudo-lymphatic " tissue has increased in bulk, and the rudiments of the mesonephros are larger than in the preceding stage. They are segmental structures beginning in the twenty-fifth segment, i. e., in the region of the posterior half of the archinephric duct, the anterior half being quite free from mesonephric tubule rudiments. They consist of small, solid knob-shaped masses of deeply staining cells quite readily distinguishable from the surrounding " pseudo-lymphatic " tissue lying mostly dorsal to the archinephric duct. Although they seem to develop outwards from the immediate neighbourhood of the duct, indenting its otherwise straight course, yet they are not formed from the cells of the duct, but appear to be condensations of mesenchyme. They rapidly increase in size, become moulded into solid twisted structures of the nature of solid cords, and eventually acquire a lumen secondarily. They extend backwards to the commencement of the urinary bladder, through eighteen segments, i.e., to the forty-third post-occipital myotome.

Stage 32.—The mesonephric tubules have become hollow in most cases, and the cells surrounding the lumen have a

more epithelial character. The slightly club-shaped end of each tubule is hollowed out into a rounded cavity very like that seen in the development of the pronephros, and a mass of darkly stained mesenchyme in the neighbourhood of each of these Malpighian capsule rudiments, probably represents the rudiment of the glomerulus.

Stage 33.—Many mesonephric tubules have acquired an opening into the archinephric duct which is distinguishable from the former by its paler staining powers. The Malpighian capsules in many of the segments are fully formed—the glomerular artery arising from the dorsal aorta by a common root with an intersegmental artery (Pl. 4, fig. 27).

Stage 34.—Horizontal sections at this stage show the segmental character of the mesonephros at its best. By this time all the mesonephric rudiments have fully developed and Malpighian capsules with their glomeruli and tubules occur, one in each segment. The urinary bladder has lengthened out and the pseudo-lymphatic tissue has increased.

Stage 35.—Although externally the creature is quite adult in appearance the mesonephric tubules have not yet attained their complete development. They are still readily distinguishable from the duct by their staining reaction, the cells of the duct being much redder in preparations stained with eosin and hæmotoxylin than the purple ones of the tubules.

The very much coiled anterior end of the duct is embedded in a capsular structure formed of " pseudo-lymphatic " tissue, the elements of which are smaller and more densely packed than in the earlier stages. Posteriorly the tissue has to make way for the tubules, and is not so abundant.

Stage 36.—The epithelial lining of the tubules has now quite an adult appearance. New secondary tubules are beginning to form between the earlier tubules. These new rudiments repeat the developmental history of the first formed units, being closely packed, densely stained tissue masses in the immediate neighbourhood of the duct when they first appear. Eventually they become coiled tubules. However,

it is very difficult to follow their history completely. These secondary tubules are not numerous in Symbranchus.

Stage 43.—The pronephric chambers with their glomeruli are still present, though insignificant structures, very much crushed out by the surrounding tissues. Their position is more posterior relative to the shoulder girdle. New meso-nephric tubule rudiments are still being formed.

The urinary bladder reaches anteriorly almost to the com-mencement of the rectum, and posteriorly a blind sac-like dilatation protrudes into the tail.

The Adult.—A dissection of the excretory system of an adult of twelve inches shows an elongated paired kidney lying on either side, and in close contact with the inter-renal vein. It extends from a point lying dorsal to the middle of the pericardium—to the rectum—and is flattened dorso-ventrally. At the beginning of the rectum (thirty-eighth trunk-seg-ment) it opens into the urinary bladder, which has the same diameter as the rest of the excretory organ. A small diver-ticulum of the bladder passes into the tail. The urinary opening is distinct from, but immediately posterior to, the anus.

(c) Vascular System.

Rudiments of the dorsal aorta appear in Stage 19 as clear spaces lying between the nephrotomes and notochord. In Stage 21 the dorsal aorta, in which blood-corpuscles are be-ginning to appear, is still paired throughout its length.

A sagittal section through Stage 22 shows that the blood from the yolk collects into a widened receptacle and then passes into a single and almost straight elongated vessel which expands somewhat just where it comes into connection with the first two visceral arches. The widened receptacle, the rudiment of the atrium, is quite anterior to the embryo. The rudiment of the ventral aorta communicates with that of the dorsal aorta by vessels which run up the mandibular and hyoid arches respectively. The blood reaches the yolk-sac

by the subintestinal vein, a large vein which is situated in the ventral part of the mesenchymatous tissue underlying the alimentary canals (*s. i. v.*, Pl. 4, fig. 25). This vein first appears in that part of the "tail" which is nearest the yolk-sac, and gradually extends backwards as the "tail" elongates.

Stage 24.—The straight tubular heart is twisting and the rudiment of the bulbus first appears. The ventral aorta is exceedingly short and topographically posterior to the ventricle, on account of the position of the pericardial rudiment. (Pl. 1, fig. 11, shows the ventricle.) The vessels in the branchial arches are making their appearance, though not yet connected up with the ventral aorta.

The anterior cardinals, ducts of Cuvier, and veins of the fin are appearing, in the form of clear spaces in the mesenchyme provided in some cases with blood-corpuscles, while in the tail a caudal vein is visible. This caudal vein some little distance anterior to the anus divides up into two branches, one of which forms the inter-renal vein, and dies out anteriorly at this stage; the other, descending to the right of the alimentary canal, forms the subintestinal vein already mentioned (*s. i. v.* Pl. 1, figs. 9, 10, and Pl. 2, fig. 13). This subintestinal vein breaks up in the yolk into a number of capillaries which drain into two large vitelline veins, which in turn form by their union the sinus venosus.

Two large vessels are given off to the pectoral fins from the dorsal aorta in the neighbourhood of the pronephros. Posterior to the pronephros, the dorsal aortæ, until now distinct, are fusing up to form a single vessel.

Stages 25 and 26 (Text-fig. 2).—The bulbus lies immediately ventral to the first branchial arch, so that the first branchial artery has a vertical direction, and the ventral aorta must take a posterior direction in order to supply the three other branchial arches. This backwardly directed part of the ventral aorta is paired. A smaller branch from the bulbus, the remnant of the disappearing hyoid aortic arch, runs forward to the hyoid arch, but it is quite insignificant compared with the four aortic arches, which practically convey all the

blood from the heart to the dorsal aorta. The mandibular
aortic arch has completely disappeared. The anterior ends
of the aortic roots are prolonged forward to the head as dorsal
carotids, and each of these is joined by a vessel from the
hyoid arch. A cœliaco-mesenteric artery is present, as has
already been explained in connection with the excretory
system.

TEXT-FIG. 2.
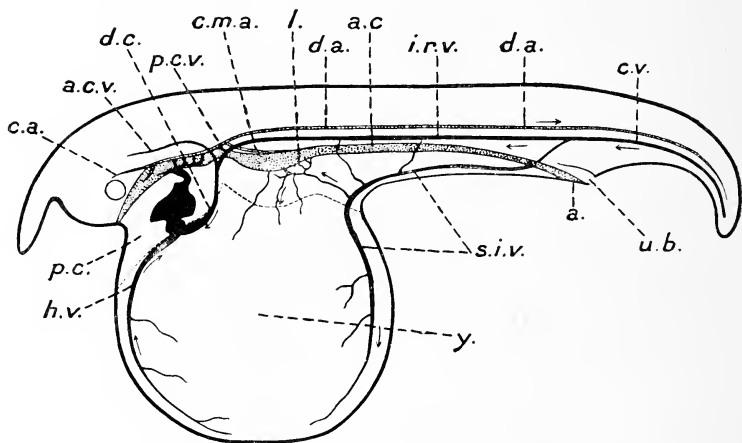
Diagram of the vascular system of a larva of Stage 26. *a.* Anus.
a. c. Alimentary canal. *a. c. v.* Anterior cardinal vein. *c. a.*
Carotid artery. *c. m. a.* Cœliaco-mesenteric artery. *c. v.* Caudal
vein. *d. a.* Dorsal aorta. *d. c.* Ductus Cuvieri. *h. v.* Vitel-
line vein. *i. r. v.* Inter-renal vein. *l.* Liver. *p. c.* Pericardium.
p. c. v. Posterior cardinal vein. *s. i. v.* Subintestinal vein. *u. b.*
Urinary bladder. *y.* Yolk.

At this stage the anterior cardinals are fully developed.
The ductus Cuvieri are long, since the sinus venosus is still
topographically the most anterior part of the heart. The
vitelline vein enters the sinus anterior to the ductus Cuvieri.
The paired portions of the posterior cardinals, now fully
formed, are very short, the inter-renal vein commencing in the
seventh segment. Posteriorly, especially in the neighbour-
hood of the developing urinary bladder, however, the inter-
renal vein shows its double nature. Blood from the yolk-

mass bathes the tissue of the liver, which, as regards its cir-
culation, may be considered as part of the yolk. The large veins
from the fin become connected up with the ductus Cuvieri.

Stage 26.—The hyoid aortic arch has almost disappeared.
The four branchial aortic arches are large. The heart is
becoming relatively more posterior and more twisted. There
are many connections between the inter-renal vein and the sub-
intestinal vein, which do not show any segmental arrangement.
Inferior jugulars now appear, and the right posterior cardinal
is becoming larger than the left one.

Stages 27 and 28.—The heart is now in such a position
that the bulbus lies under the third gill-cleft, so that the
ventral aorta has to run forwards to the first and second
afferent branchial arteries and backwards to the third and
fourth. A small branch of the ventral aorta to the hyoid
arch still persists. In Stage 30 this has disappeared. The
aortic roots join up to form the dorsal aorta posterior to the
fourth branchial arch, the fourth branchial efferents being
almost at right angles to the dorsal aorta. Anteriorly the
aortic roots are continued forwards as the dorsal carotids,
which receive the efferent end of the hyoid aortic arches.
Near the infundibulum the carotids divide up into internal
and external branches, which are distributed, the former to
the brain, the latter mainly to the eyes and nose. The
inferior jugulars which collect the blood from the lower jaw
and the parts below the ventral aorta, and which are asym-
metrical, the right being smaller than the left, pass between
the bifurcating anterior ends of the thyroid. The ventricle is
now topographically the anterior part of the heart.

Stage 29.—Text-fig. 3A shows the principal veins at this
stage and needs no description.

Stage 30.—The vascular system is practically in its adult
condition at this stage. Sinus venosus, atrium, ventricle and
bulbus have assumed their adult topographical relations,
though all these structures are not relatively so elongated as
in the adult Symbranchus, nor does the heart occupy such a
posterior position. The ventral aorta, moreover, is not the

long structure it is in the adult. The heart shows the ordinary Teleostean features (compare Pl. 4, fig. 26). An

TEXT-FIG. 3.
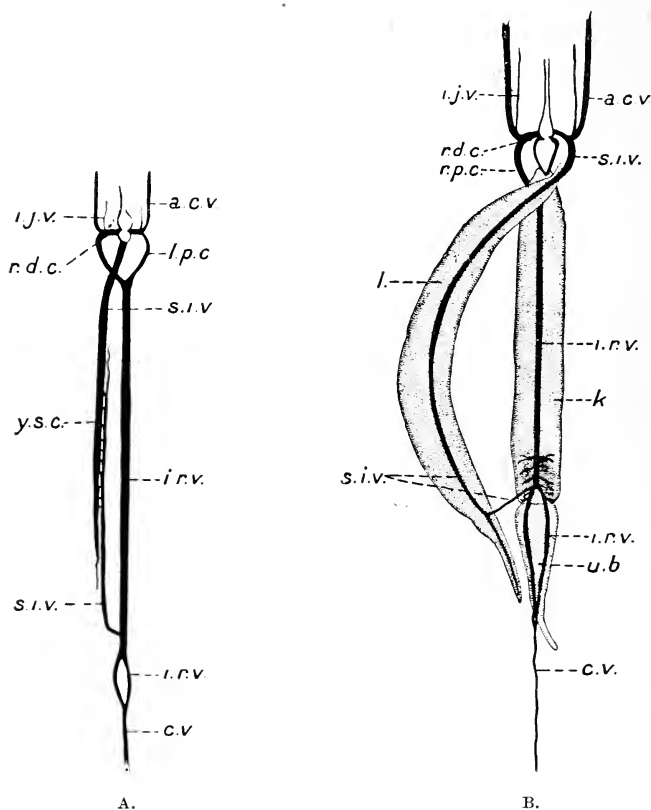
A. Diagram of the principal veins of a larva of Stage 29 (ventral view). B. Diagram of the venous system of adult Symbranchus (ventral view). The liver has been pinned out to the right. *a. c. v.* Anterior cardinal vein. *c. v.* Caudal vein. *i. j. v.* Inferior jugular vein. *i. r. v.* Interrenal vein. *k.* Kidney. *l.* Liver. *l. p. c.* Left posterior cardinal. *r. d. c.* Right ductus Cuvieri. *r. p. c.* Right posterior cardinal. *s. i. v.* Subintestinal vein— vitelline vein—hepatic vein. *u. b.* Urinary bladder. *y. s. c.* Yolk-sac circulation.

artery is given off from the dorsal aorta to supply the urinary bladder. Intersegmental arteries are also given off to

the body-segments from the dorsal aorta (Pl. 4, fig. 27), while segmental veins convey venous blood from the muscles into the blood sinus which bathes the excretory organs. The inter-renal vein is double in the neighbourhood of the urinary bladder, this structure lying between the two branches; the right blood-vessel is always the larger.

The free portion of the left posterior cardinal (compare Text-fig. 3A) is reduced in size at this stage; eventually it atrophies completely, the blood of the inter-renal vein being conveyed to the heart by the right posterior cardinal. The vitelline vein, instead of opening ventrally into the sinus venosus, has its opening shifted well to the left so that it opens into the ductus Cuvieri symmetrically with the large right posterior cardinal (Text-fig. 3B). It has been shown in the earlier stages that the liver is practically a part of the yolk as regards its circulation. It has also been shown that the caudal vein is continued forwards as an inter-renal (which, however, is a paired vessel in the neighbourhood of the urinary bladder) and a subintestinal vein. This division of the caudal vein takes place at the anterior end of the urinary bladder. In the earlier stages the subintestinal vein breaks up in the yolk into a number of capillaries which unite to form a large vitelline vein. However, as the creature grows, and as more and more of the yolk-mass becomes included in the normal contour of the animal, the subintestinal vein never loses its individuality, so to speak, but can be traced forwards through the liver, becoming the hepatic vein. This vein gradually increases in diameter as the pericardium is reached, because it receives blood from the yolk and liver. As the creature grows and as the liver elongates blood communications between kidney and liver are also set up. This is very striking in Stage 30, when the subintestinal vein gives off a large branch, which, running ventral to the alimentary canal, enters the posterior end of the liver and there breaks up into capillaries. Later on more blood passes into the liver from the hinder parts of the creature, this blood eventually finding its way into the anterior parts of the sub-

TEXT-FIG. 4.
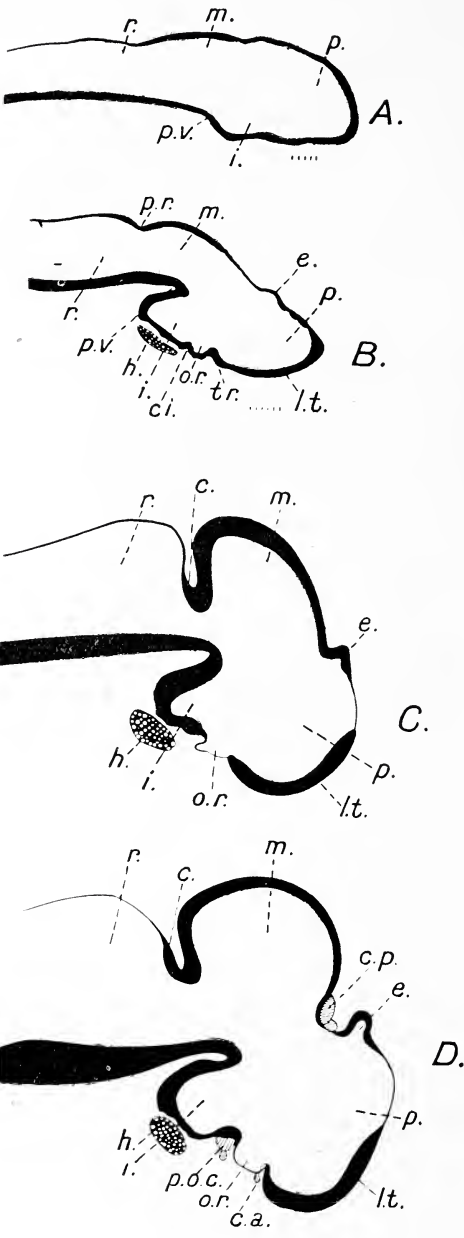
intestinal vein, the front end of which is the original vitelline vein.   Even when the yolk has quite disappeared this large subintestinal vein, can be traced right up to the liver.   The

TEXT-FIG. 4—continued.
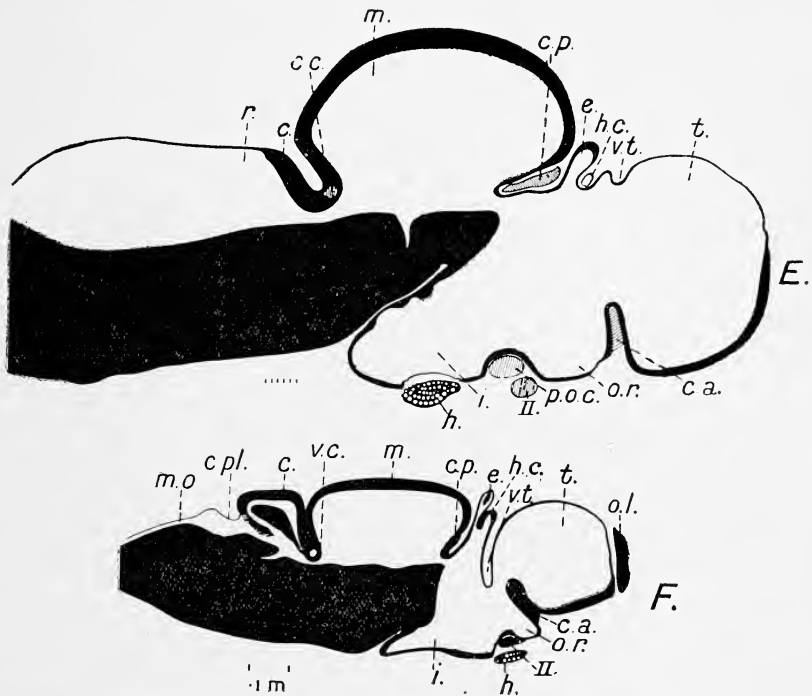
Sagittal sections through brain of Symbranchus. A. Stage 22. B. Stage 24. c. Stage 26. D. Stage 28. E. Stage 30. F. Stage 34. *c.* Cerebellum. *c. a.* Commissura anterior. *c. c.* Cerebellar commissure. *ci.* Optic chiasma. *c. p.* Commissura posterior. *c. pl.* Choroid plexus. *e.* Epiphysis. *h.* Hypophysis. *h. c.* Habenular commissure. *i.* Infundibulum. *l. t.* Lamina terminalis. *m.* Mesencephalon. *m. o.* Medulla oblongata. *o. l.* Olfactory lobes. *o. r.* Optic recess. *p.* Prosencephalon. *p. o. c.* Post-optic commissure. *p. r.* Rhombo-mesencephalic fissure. *p. v.* Primitive brain fold. *r.* Rhombencephalon. *t.* Telencephalon. *tr.* Torus transversus. *v. c.* Valvula cerebelli. *v. t.* Velum transversum. II. Optic nerve.

subintestinal vein is thus seen to pass directly into the hepatic vein of the adult, and its junction with the kidney vessels posteriorly recalls the posterior vena cava in Polypterus. (6).

(D) Nervous System.

The Brain.

The course of events in the conversion of the flat, wide-spreading medullary plate into the solid keel-like neural rudiment, and the formation, out of the anterior part of this plate, of a brain-rudiment with ventricles which develop secondarily, is quite Teleostean in character. Except for a slighter wider diameter and the presence of optic vesicles there is nothing to distinguish the brain from the spinal cord in the stages up to 20. In a dorsal view of Stage 22, however, the three ventricles are discernible, while sagittal sections of about this age (Text-fig. 4 A) show that the floor of the archencephalon is becoming delimited posteriorly by an uprising of the brain floor.

Cranial flexure has increased considerably by Stage 24 (Text-fig. 4 B), when the ventricles are very well defined. The mesencephalon and rhombencephalon are conspicuous objects in a dorsal view of the whole embryo (Pl. 1, fig. 7), a somewhat narrow portion connecting the latter with the former. Indications of a cerebellum rudiment are discernible, as two thickenings on the anterior wall of the rhombencephalon cavity. In the prosencephalon a pineal rudiment is distinguishable. The optic recess is somewhat narrow.

Cranial flexure continues to increase (Text-fig. 4 c), reaching a maximum in Stage 28 (Text-fig. 4 D). By this time (Stage 28) nerve-fibres have appeared. The posterior commissure and habenular ganglia as well as anterior and post-optical commissures are formed. The paired thickenings alluded to above have grown backwards and inwards to form a rudimentary cerebellum, while on either side of the lamina terminalis the basal thickenings are converting the prosencephalon into a solid structure with a thin non-nervous roof.

By Stage 30 (Text-fig. 4 E) the brain has begun to straighten out and to assume much of its adult appearance in transverse sections. The rudiment of the velum transversum is present

and olfactory lobes are developing. The infundibulum is closely apposed to the floor of the hind brain, which latter is becoming very solid. In addition to the commissures already present the cerebellar commissure has now appeared, a torus longitudinalis is distinguishable, and the fibres of the optic nerve are connected up with the roof of the mid-brain, as is usual.

There is no fundamental difference between the brain of Stage 34 (Text-fig. 4 F) and that of the adult. The brain is by now (Pl. 4, fig. 28) very solid, the once large ventricles being almost obliterated. The paired olfactory lobes are conspicuous at the anterior end of the telencephalon, while ventrally the lateral lobes of the infundibulum have greatly increased in size. The cerebellum and valvula cerebelli are also much larger, while the tuberculum acusticum and the vagus lobes have almost obliterated the once large fourth ventricle.

The brain dissected from an adult Symbranchus is characterised by a more perfect separation of the chief brain parts one from the other. These are very close together in Stage 34. Another difference is that the optic lobes are not relatively so large as they were. The telencephalon, on the contrary, is larger, while the cerebellum and valvula cerebelli are much more important than they were at Stage 34. The sacculus also develops late.

Cranial Nerves.

The cranial nerves of the adult Symbranchus as well as their respective branches are quite typical.

The eye-muscle nerves are small, like the eye itself, and the olfactory and optic nerves are long because of the general elongation of the head.

The developmental history of the cranial nerves is, briefly, as follow:

Stage 21.—The rudiments of the ganglia of the fifth, seventh and tenth nerves are visible.

Stage 24.—Nerve-fibres have appeared in the peripheral parts of the brain and the spinal cord, and the roots of the fifth, seventh, eighth, ninth and tenth can be distinguished. These roots are quite distinct one from the other.

Stage 25.—The large roots of the fifth, seventh, eighth, ninth and tenth are conspicuous objects in sections, the fifth and seventh, in contra-distinction to their adult condition, being separated by a considerable interval. The optic and olfactory nerves are possessed of fibres.

Stage 26.—The third nerve is developing. On account of the cranial flexure this nerve appears before the optic nerve in transverse sections.

Stage 28.—The fourth nerve can be traced from the junction of the optic lobes with the rhombencephalon. The acustico-lateral ganglionic elements of the seventh nerve can be easily distinguished from the Gasserian ganglion in these early stages and the lateral line root is widely separated from the vagus root. Even in the adult these two roots are separate, the lateral line branch transversing part of the floor of the auditory capsule in order to reach the vagus ganglion.

The three main branches of the fifth can be traced to their respective destinations in Stage 28 and the hyomandibular branch of the seventh is conspicuous. The rudiments of the pre- and post-trematic branches of the ninth and tenth nerves are distinguishable in sagittal sections, as well as the palatine branch of the ninth.

Stage 30.—Gasserian and geniculate ganglia are becoming massed into one large ganglionic centre which lies outside the brain-capsule—the condition which obtains in the adult. The pre- and post-trematic branches have fibres. By Stage 32 buccal and palatine branches of the seventh as well as ophthalmic can be traced.

Spinal Nerves.

Fibres can be detected in the spinal nerve-rudiments in Stage 24, although the nerve is mostly protoplasmic at this

stage. The nuclei, which later on will form the nerve-sheath, lie more or less scattered round this protoplasmic-like developing nerve.

In Stage 26 these nerve-sheath nuclei, which have been derived from the mesenchyme separating spinal cord from myotome, are more closely apposed to the nerve-rudiment, which is now much more strongly fibrous and has almost entirely lost its protoplasmic character. In stages beyond 26 the nerves have the usual adult structure.

IV. Summary.

(a) General Features of Development.

(1) The egg of Symbranchus is small, its development typically Teleostean and rapid, the larva hatching out in about seven days at a tropical temperature.

(2) A rostrum appears just before the larva hatches, increases in size, attains a maximum length of about 1 mm. when the creature is 7 mm. long, decreases in size, gradually dying down to a rounded pad, and eventually disappears just before the adult stage is reached.

(3) The larva possesses pectoral fins and the shoulder girdle persists in the adult. These fins appear early, are muscularised by the first three trunk myotomes and innervated by the first three spinal nerves. They develop rapidly, reach their maximum size seven or eight days after hatching, shrivel somewhat, and then drop off bodily at Stage 34. The pectoral fins are mainly respiratory organs and possess a rich network of capillaries. There are three principal blood-streams in the fins—one central, afferent, two marginal, efferent.

The establishment of perfect branchial respiration is co-incident with the falling off of the fins, i. e. when the creature is ten days old.

(4) No trace of pelvic fins has been found.

(5) Perforated gill-slits of the Elasmobranch type do not occur in early stages, the clefts only becoming perforate after

they are covered by the operculum. When branchial respiration is just beginning the gill-chamber opening is a single crescent-shaped one; as development proceeds the arms of the crescent are gradually obliterated, owing to the fusion of the backwardly growing operculum with the body-wall, and a single median ventral opening is the result.

(6) There is a blind diverticulum in the dorsal roof of the mouth behind the hyoid.

(B) Alimentary Canal.

(1) The alimentary canal has a typical Teleostean character and development, is solid at first, hollowed out secondarily, and has no obvious connection with the yolk.

(2) No air-bladder has been detected at any stage.

(3) The pyloric valve arises by outpushings of the intestine. These blind cæcal outgrowths have the appearance of very short rudimentary pyloric cæca.

(4) Apart from these structures there are no pyloric cæca.

(5) The pancreas is an elongated compact gland arising from a dorsal and two ventral rudiments.

(6) The liver is elongated and unilobed.

(7) There is a typical thymus arising from clefts 2, 3, 4 and 5.

(8) A thyroid arises as a solid median derivative of the floor of the pharynx. It is elongated and bilobed anteriorly.

(9) The spleen develops early, is very conspicuous, and multilobed at first.

(c) Renal Organs.

(1) The pronephric chamber and tubule are formed from the nephrotome of the third trunk myotome.

(2) There is no communication at any time between splanchnocœle and nephrocœle of the pronephros.

(3) The archinephric duct is formed from the nephrotomes of the segments posterior to the third; the conversion of these nephrotomes into a duct takes place simultaneously, involving no backward growth of the archinephric duct.

(4) The pronephros is still present in the oldest larva examined.

(5) Mesonephric tubule-rudiments appear in Stage 29. They occur from about Segment 25 to Segment 43. Each arises as a rounded clump of darkly stained cells in the immediate neighbourhood of the archinephric duct. This rudiment is gradually moulded into a twisted tubule, one end of which becomes converted into a Malpighian capsule of the usual type, the other end acquiring an opening into the archinephric duct.

(6) There are no peritoneal funnels.

(7) Secondary mesonephric tubules arise in connection with the archinephric duct and with the primary mesonephric tubules. These are not fully differentiated in the oldest larva examined.

(8) The anterior much-coiled part of the archinephric duct, as well as the mesonephros, is surrounded by pseudo-lymphatic tissue.

(D) Vascular System.

(1) The development of the heart and vascular system agrees generally with that described for other Teleosteans.

(2) The free anterior part of the left posterior cardinal disappears, the large right posterior cardinal conveying the blood of the inter-renal vein to the heart.

(3) There is a close connection between the blood-vessels of the hinder ends of the kidney and liver recalling the posterior vena cava of Polypterus.

(4) The subintestinal vein, the front end of which is the vitelline vein of the earlier stages, persists in the adult as a hepatic vein. This hepatic vein joins up with the left anterior cardinal and left jugular to form the left ductus Cuvieri. The right ductus Cuvieri shows no special peculiarity.

(e) Nervous System.

(1) The brain is at first solid and is hollowed out secondarily.

(2) Three main divisions of the brain can be distinguished in Stage 21.

(3) There is no cranial flexure until Stage 24, and therefore no reason for assuming that the infundibulum is the morphologically anterior end of the brain.

(4) Sagittal sections through the brain at different stages show the usual Teleostean characters.

(5) The cerebellum is late in developing and goes on growing after metamorphosis.

(6) The optic lobes of the mature brain are relatively smaller than in the developing one. The mid-brain of the adult is the least conspicuous part.

(7) The mature brain is elongate, as also are the olfactory and optic nerves, the divisions well separated off.

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EXPLANATION OF PLATES 1—4.

Illustrating Miss Monica Taylor's paper on "The Development of Symbranchus."

PLATE 1.

Fig. 1.—A surface view of the youngest stage in the collection. The segmenting blastoderm looking like a white circular pad "let into" the yolk-mass occupies the centre of the flattened pole of the egg.

Fig. 2.—An egg of Stage 10. The blastoderm has a thickened rim, and at one point this rim becomes accentuated to form the embryonic rudiment.

Fig. 3.—An egg of Stage 11.

Fig. 4.—An egg of Stage 17.

Fig. 5.—An egg of Stage 21. *o. v.* Optic vesicles. *p. f.* Pectoral fins. *v. r.* Rudiment of visceral arches with their nerve supply.

Fig. 5A.— An egg of Stage 21, fixed in corrosive acetic. *b. r.* Branchial rudiment. *h. a.* Hyoid arch. *m. a.* Mandibular arch. *o. v.* Optic vesicles. *p. f.* Pectoral fins.

Fig. 6.—An egg of Stage 22.

Fig. 7.—An embryo of Stage 23. *c. s.* Cartilaginous skeleton of pectoral fin. *e.* Eye. *m.* Mesencephalon. *o.* Otocyst. *r. c.* Rhombencephalon. *r.* Rostrum.

Fig. 8.—Side view of an embryo of Stage 24. *e.* Eye. *n.* Nostril. *o.* Otocyst.

Fig. 9.—Larva of Stage 26. *a.* Anus. *c. r.* Cerebellum rudiment. *e.* Eye. *h.* Heart. *n.* Olfactory organ. *o. l.* Optic lobes. *op.* Operculum. *s. i. v.* Subintestinal vein.

Fig. 10.—A larva of Stage 30. *s. i. v.* Subintestinal vein. *y.* Yolk.

Fig. 11.—Sagittal section through an embryo of Stage 25. *i.* Inner and *o.* outer layer of ectoderm. *e.* Epiphysis. *m.* Mesencephalon. *pc.* Pericardium. *p.* Prosencephalon. *r.* Rhombencephalon. *v.* Ventricle.

PLATE 2.

Fig. 12.—Right pectoral fin and shoulder girdle of a larva of Stage 30.

Fig. 13.—A larva of Stage 32. *s. i. v.* Subintestinal vein. *y.* Yolk.

Fig. 14.—Ventral view of larva of Stage 33. *o. o.* Opercular opening.

Fig. 15.—A larva of Stage 33. *o. o.* Opercular opening.

Fig 16.—Stage 35.

PLATE 3.

Fig. 17.—Paraffin sagittal section through an egg, Stage 8. *s.* Space underlying blastoderm. *y. s.* Yolk syncytium.

Fig. 18.—A celloidin sagittal section, 25 μ thick, through an egg of Stage 10. *ec.* Ectoderm. *en.* Endoderm. *n. p.* Neural plate. *y. s.* Yolk syncytium. *y. s. n.* Yolk syncytium nuclei.

Fig. 19.—Transverse section through a larva of Stage 32. The operculum has grown backwards to the point of origin of the pectoral fins. *a. c. v.* Anterior cardinal vein. *d. a.* Dorsal aorta. *i. j.* Inferior jugular. *o.* Operculum. *s. a.* Subclavian artery. *s. g.* Shoulder girdle. *s. v.* Subclavian vein.

Fig. 20.—A horizontal section through a larva of Stage 25. *cœ.* Cœlom. *g. c.* Gill-chamber. *l.* Liver. *m.* Mesencephalon. *p. f.* Pectoral fin. *o.* Operculum. I, II, III, IV. Branchial arches 1–4.

Fig. 21.—A transverse section through a larva of Stage 26. *a. c.* Alimentary canal. *a. d.* Archinephric duct. *b. d.* Bile-duct. *cœ.* Cœlom. *d. a.* Dorsal aorta. *d. p.* Dorsal pancreas. *l.* Liver. *s.* Spleen. *y.* Yolk.

Fig. 22.—Sagittal section through a larva of Stage 34. The figure showing the brain is drawn from one section, that showing the lower jaw from another section, the distance between the two sections being 20 μ. *a. o.* Olfactory organ. *c.* Cerebellum. *c. pl.* Choroid plexus. *c. g.* Gill-cavity. *h. g.* Habenular ganglion. I. Infundibulum. *ol. l.* Olfactory lobes. *o. l.* Optic lobe. *thy.* Thyroid. *t.* Tongue. *v. t.* Velum transversum. II. Optic nerve.

PLATE 4.

Fig. 23.—Transverse section through a larva of Stage 29. *a. c. v.* Anterior cardinal vein. IV. *ef. bra.* Fourth efferent branchial. III, IV, *g. a.* Third and fourth branchial arch. *g. c.* Gill cavity. *p.* Pericardium. *p. o.* Pore-like opening into gill-chamber. *y. s.* Yolk-sac.

Fig. 24.—Transverse section through a larva of Stage 35. *d. a.* Dorsal aorta. *g. b.* Gall-bladder. *i. r. v.* Inter-renal vein. *l.* Liver. *plt.* Pseudo-lymphatic tissue surrounding kidney. *p. v.* Pyloric valve. *p. a.* Pancreas. *s.* Stomach (pylorus). *s. i. v.* Hepatic vein.

Fig. 25.—Sagittal section through an embryo of Stage 24. *a. c.* Alimentary canal. *d. a.* Dorsal aorta. *n.* Notochord. *s. c.* Spinal cord.

s. i. v. Sub-intestinal vein. *y. s.* Yolk syncytium. *y. s. n.* Yolk-syncytium nucleus.

Fig. 26.—Sagittal section through a larva of Stage 32. *a.* Atrium. *b.* Bulbus. *a. c.* Alimentary canal. *s. v.* Sinus venosus. *v.* Ventricle. *v. v.* Vitelline vein = sub-intestinal vein = portal vein. *y. s.* Yolk-syncytium.

Fig. 27.—Transverse section through a larva of Stage 33. *a. c.* Alimentary canal. *d. a.* Dorsal aorta. *g.* Glomerulus. *g. a.* Glomerular artery. *i.r.v.* Interrenal vein. *ia.* Intersegmental artery. *k. t.* Kidney tubules. *l.* Liver. *n.* Notochord. *y.* Yolk.

Fig. 28.—Brain dissected from a larva of Stage 34. A. Dorsal; B. Ventral view. *c.* Cerebellum. *l. l.* Lateral lobes of infundibulum. *m. o.* Medulla oblongata. *o. l.* Olfactory lobes. *t.* Telencephalon. *th.* Thalamencephalon. *t. o.* Tectum opticum.

The Development of the Heart and Vascular System of Lepidosiren paradoxa.

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With Plate 5 and 31 Text-figs.

Contents.

I. Introduction.

In the following account of the development of the heart and blood-vessels of Lepidosiren paradoxa I have endeavoured to trace their main morphological rather than their minute anatomical relations. I trust that few points of importance have been neglected, though probably many minutiæ of interest have been passed over.

The account of the adult condition has been obtained partly from the dissection of one complete and one bisected individual (cranial half), whose vessels were injected with a gelatine mass at air temperature on a hot tropical afternoon (the vessels were first washed out with normal saline solution, nitrite of amyl being administered to keep the arterioles dilated), and partly from the dissection of a dozen uninjected adult heads, which included the cardiac region and heart. This account only includes the more obvious details that could be ascertained by a conservative dissection of the injected material mentioned. The account of the development of the vascular system has been obtained from the scrutiny of serial sections in three planes of a complete series of embryos from Stage 23—when the vessel rudiments first appear—to Stage 38, when the adult condition in all but size has been attained. The finer morphological and anatomical relations of the adult, therefore, have been noted in conjunction with the examination of the development of the various parts of the vascular system, Stage 38 of the sectional material being considered as equivalent to the adult condition. The portal system, it will be noticed, is reported only in the notes on the development of the venous system, as it was considered unnecessary to submit the valuable injected specimens to the serious disturbance involved by the dissection of the vessels of that system. The study of the development of the heart was further facilitated by the dissection under the Zeiss binocular dissecting microscope of embryos of varying ages.

Throughout these notes I have called the distal arterial segment of the heart the bulbus cordis. I am aware that it is still a matter of debate whether this region of the heart is more correctly entitled conus (8) or bulbus, but meantime, till a final solution of the question is reached, I have preferred to employ a nomenclature that brings this account more easily into line with the work of Langer (16) and Greil (9).

I have to express my great indebtedness to Professor Graham Kerr for free access to all his valuable Lepidosiren

material and for much helpful criticism throughout the course of my work.

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II. Summary of General Features of the Vascular System of Lepidosiren.

The main points of interest in the comparative anatomy of the heart and vascular system of Lepidosiren are here briefly summarised. Further references will be found in the text in the course of the article itself.

The heart of Lepidosiren is distinguished by the presence of well-marked, though incomplete, auricular, ventricular and bulbar septa, the absence of any pocket or semilunar valves, and the separate opening of the pulmonary vein into the left auricle. Two other distinctive features are the long, abruptly curved bulbus cordis, and the single button-like plug that acts as an auriculo-ventricular valve. The heart of Lepidosiren most closely resembles that of Protopterus (2), but differs from it in possessing definite, though incomplete, interauricular and interventricular septa. Compared with the heart of Ceratodus (2) and (14), the main points of difference are: (1) The more abrupt kinking of the bulbus cordis; (2) the almost complete disappearance in Lepidosiren of all the bulbar pocket valves as such, except proximally, where a few vestigial valves still persist (17); (3) the development of the spiral valve as a structure that extends throughout the length of the bulbus; (4) the presence of a longitudinal valve on the left wall of the distal part of the bulbus; (5) the shortening of the posterior auricular wall, so that in Lepidosiren the sinu-auricular and auriculo-ventricular openings are closely approximated. In Lepidosiren the ventricle is comparatively a larger part of the heart, and the interventricular septum is much more definitely a septal structure, though its attachments appear to be identical;

while the auriculo-ventricular plug or fibro-cartilage is
apparently better adapted for closing the auriculo-ventricular
opening than the similar structure in the heart of Cera-
todus. In short, the heart of Lepidosiren, as compared
with that of Ceratodus, shows a marked advance in the
development of a mechanism for the more complete sepa-
ration of the arterial and venous blood-streams.

The general facts of the development of the heart are
comparable with those described for the Elasmobranchs (12)
in so far as the division into different chambers is concerned;
the details, however, differ considerably. In Lepidosiren
the auricular canal is only transitorily a distinct external
division of the heart, and early loses its identity in the
auriculo-ventricular opening. The posterior auricular wall
remains very short and the sinu-auricular and auriculo-ventri-
cular openings are consequently close together. The auriculo-
ventricular plug, which is apparently a peculiarly dipnoan
structure and which seems to be identically developed in
Ceratodus (14), can, however, be compared in origin and
function both to the posterior (dorsal) auriculo-ventricular
valve of the Elasmobranchs, and to the posterior (dorsal)
sinu-auricular valve in Lepidosteus (2), but at no period is
there any equivalent to an anterior (ventral) valve. The right
sinu-auricular valve develops as the similar structure in the
Elasmobranchs, but it is often very poorly developed and
there is no left valve. The pulmonary vein develops in the
left wall and roof of the sinus venosus, and opens, from the
first, directly into the left auricle as in the reptilian heart (21),
the auricular connection of this vein in the Urodele being a
secondary, not a primary condition. The interauricular
septum of Lepidosiren develops in the same way as that
of the Urodeles (12), except that the termination of the pul-
monary vein projects somewhat into the auricles in the
former, and actually forms the most posterior part of the
septum. The development of muscular trabeculæ in the
ventricle proceeds as in all the Vertebrata; similarly they
extend into the auricular canal and become continuous with

the valvular apparatus, the auriculo-ventricular plug, situated there. This structure, however, with its thick, centrally placed muscular attachment on its ventral surface, does not become hollowed out to form a pocket valve, but gives rise to the typically dipnoan auriculo-ventricular plug. The development of the long bulbus cordis resembles in some degree that of the same division of the heart in the Elasmobranchs, e.g. in the appearance for a time during development of four endocardial cushions in the distal and proximal parts of the bulbus, and the persistence proximally in the adult of three rows of vestigial pocket valves, as well as of transverse furrows at the base of the spiral valve. The discontinuous development of the spiral valve in two segments can be compared with the similar condition in Urodeles (16). The heart of Lepidosiren therefore presents different elasmobranch, amphibian, and even reptilian, as well as some peculiarly dipnoan characters.

Some of the main points of interest in the arterial system are the development of six aortic arches, and the close resemblance of their ultimate arrangement to that present in the Urodeles (3), the lingual and dorsal carotid arteries of Lepidosiren corresponding to the external and internal carotids of the former. The pulmonary arteries are developed directly in connection with the sixth aortic arches, and come to open secondarily from the dorsal aortic roots; whereas in the urodele they are tending to become more direct continuations of the sixth aortic vessels. The development of the cœliac artery as primarily the right glomerular vessel is comparable with the teleostean condition, but its lack of further connections with any other vessels except in the wall of the gut appears to be a detail peculiar to Lepidosiren.

In the venous system the development of the anterior cardinal veins tallies generally with that of the Elasmobranchs and amphibians, while that of the posterior cardinal veins agrees with the amphibian type, except that in Lepidosiren the fused interrenal portions of the two vessels become separated again. The anterior section of the posterior vena

cava develops centripetally towards the heart, and is to be
looked on as a short cut connecting the large right posterior
cardinal vein with the heart.   Both posterior cardinals
persist in the adult, the left in its entirety, the right only as
a comparatively short vessel.   The development of the sub-
intestinal and portal veins is essentially Elasmobranch (20) in
character.

    The vascular system of Lepidosiren would thus appear
to have many points in common with both the Elasmobranchs
and the amphibians, and to occupy a position between the two,
though tending more towards the latter.   As was to be
expected, the adult conditions are almost identical with those
described for Protopterus; some of the main points agree
with those given for Ceratodus, but the details of the
vascular system of the latter—apart from the heart—appear
to be much more closely piscine than in Lepidosiren.

The Heart and Vascular System of the Adult Lepidosiren.

(a) Heart.

    In Lepidosiren the heart is placed far forward and lies in
a thick pericardiac envelope that splits into two layers in the
lateral and ventral portions of its posterior half.   This split
forms a lymph space over the posterior part of the ventral
surface of the inner pericardium; the two pericardiac layers
come together again at the entrance of the posterior vena
cava into the sinus venosus.   The pericardium is attached to
the heart over the dorsal surface of the sinus venosus, round
the posterior vena cava as it enters the sinus, to the ventral
surface of the ventricle by a special fibrous band—gubern-
aculum cordis of Fritsch (7)—and to the anterior extremity
of the bulbus cordis.   The outer surface of the pericardium
is firmly attached at the sides to the body musculature and
anteriorly to the pectoral girdle.

    The heart as a whole is of irregular oval shape (Pl. 5, figs.

1 and 2) measuring in one large adult male $2\frac{1}{4}$ by 1 in.;
the ventricle forms little more than half the length,
the rest being due to the bulbus cordis. The heart is
fixed in the pericardiac cavity as described above; it
is almost mesial in position, and the apex is directed
backwards, a little ventrally and to the left. The heart
is wrapped in a fine sheath of lymphatics, which
invests the ventricles and auricles closely, but is looser and
more prominent over the bulbus and in the grooves between
the different cardiac compartments, the auricles in fact are
held applied to the posterior and lateral surfaces of the
bulbus by this sheathing.

Sinus Venosus.—The sinus venosus is a comparatively
large, thin-walled compartment of irregular pear shape,
situated posteriorly on the dorsal surface, and to the right
side of the heart (Pl. 5, figs. 1 and 2, *S. V.*). It is demarcated
from the auricles by a groove, especially well marked on the
right side, which is the external expression of a fold guarding
the sinu-auricular aperture (Pl. 5, fig. 1, *r. S. A.*); the roof or
dorsal wall of the sinus is continuous with the pericardium
(Pl. 5, figs. 1 and 2, *Per.*) and the pulmonary vein (Pl. 5,
fig. 1, *P. V.*) crosses it from right to left; posteriorly, the
posterior vena cava (Pl. 5, fig. 1, *P. V. C.*) opens into the
sinus and in front of this the little coronary vein (Pl. 5,
fig. 1, *C. V.*) from the dorsal wall of the right ventricle opens
on its floor. The ventral wall of the sinus rests upon
the dorsal wall of the ventricles posteriorly and upon
that of the auricles anteriorly where the two ducts of
Cuvier open into it (Pl. 5, fig. 1, *r. D. C.* and *l. D. C.*).
The apertures of these vessels are separated from one
another by the posterior border of a fold projecting
obliquely from the roof and anterior wall of the sinus and
which contains the pulmonary vein. This oblique fold
divides the anterior part of the sinus somewhat unequally
into a smaller left and a larger right compartment : the right
compartment receives in front the right ductus Cuvieri, whilst
below this, to the right of the oblique fold, is the sinu-auri-

cular orifice (Pl. 5, fig. 1); the left compartment receives
in front the left ductus Cuvieri.

The sinu-auricular aperture (Pl. 5, fig. 1) is compara-
tively large and oval in shape and opens into the right
auricle immediately to the right of the pulmonary fold (Pl.
5, fig. 1, *p. f.*).   The opening is guarded on its right side
by the vertical fold already mentioned that projects from the
region of the sinu-auricular groove (Pl. 5, fig. 1, *r. S. A.*); the
degree of development of this fold, however, varies very
much in different specimens; in one it was represented merely
by irregular thickened projections of the right rim of the
sinu-auricular opening.   A similar variability is noted for
Protopterus (**2**), Amia, Lepidosiren and Polypterus
(**21**).

Auricles.—The auricles are large, extremely thin-walled
structures which, when dilated, bulge round the bulbus cordis
and ventricles so as almost completely to surround them;
they are markedly lobed and their margins are more or less
digitate.   There is no distinct external division between the
two auricles, the situation of the interauricular septum being
indicated only by a faint groove.   The right auricle is the
larger of the two; its long anterior process is wrapped round
the ventral surface of the distal part of the bulbus cordis, a
middle or transverse process reaches the middle line of the
ventral surface of the heart at the anterior end of the
ventricles, and its lower margin reaches, in some instances,
to the apex.   Both auricles are attached round the margins of
the auriculo-ventricular aperture (Pl. 5, figs. 1 and 2), and are
held applied against the bulbus by the lymphatic sheathing
already described.   The ventral wall of the auricle and the
dorsal wall of the bulbus cordis form a sharp projecting rim
internally, and a corresponding bulbo-auricular groove exter-
nally round the anterior part of the auriculo-ventricular
opening (Pl. 5, figs. 1 and 2, *B.A. g.*)   Dorsally and to the
right the auricles are attached to the sinus venosus, but
only the right auricle communicates with that compartment.

Posteriorly, projecting into and between the auricles where

they are attached to the posterior margin of the auriculo-ventricular opening, is a fold in which the pulmonary vein reaches the left auricle (Pl. 5, fig. 1, *p. f.*) ; this fold projects across, and is attached along the dorsal surface of, the auriculo-ventricular plug and forms the posterior part of the interauricular septum. The cavities of both auricles are traversed from roof to floor by a delicate loose meshwork of muscular bands, except in the region of the auriculo-ventricular aperture ; this meshwork condenses in front of the pulmonary fold and is attached to it to complete the inter-auricular septum (Pl. 5, figs. 1 and 2, *A.S.* and *P. f.*). The meshwork passes from the anterior margin of the pulmonary fold to the ventral auricular wall (applied against the dorsal wall of the proximal part of the bulbus), arching across the narrow space between the margin of the auriculo-ventricular plug and the anterior rim of the auriculo-ventricular opening (Pl. 5, figs. 1 and 2).

The closeness and extent of this trabecular meshwork in the region of the auricular septum seems, however, to vary greatly in different specimens : in some the trabeculæ are numerous and compactly arranged, projecting considerably into the auricle, and there may be sheets of fine connective tissue between them, while in the others the trabeculæ are fewer and widely separate without any intervening connective tissue.

The interauricular septum, therefore, is composed of two elements which are joined together : a pulmonary fold pos-teriorly, and the auricular meshwork anteriorly (Pl. 5, figs. 1 and 2). This septum, however, does not ever completely shut off the two auricles from one another, there being always a space left just over the auriculo-ventricular opening.

As already noted, the pulmonary vein opens into the left auricle on the left surface of the posterior part of the inter-auricular septum (pulmonary fold), immediately dorsal to the left half of the auriculo-ventricular plug, and its opening is guarded by a hood-like fold that is attached ventrally to the anterior rim of the auriculo-ventricular plug (Pl. 5, fig. 2,

P. V. and *P. f.*).   There are no auricular valves of any kind
except the auriculo-ventricular plug.

Auricular Canal.—The auricular canal cannot be recog-
nised as a special division of the heart, and it is represented
apparently only by a narrow flattened band of musculature
on the rim of the auriculo-ventricular opening converging dor-
sally on to the auriculo-ventricular plug.   On dissection, this
musculature of the auricular canal is found to be continuous
anteriorly with the proximal part of the dorsal wall of the
bulbus cordis, and laterally with the auricular musculature
on the one hand and the ventricular on the other, round the
rim of the auriculo-ventricular opening ; posteriorly it becomes
continuous with the musculature of the interventricular septum
at the site of attachment of the auriculo-ventricular plug to
the rim of the auriculo-ventricular opening.

Ventricles.—The ventricular portion of the heart is
a thick-walled muscular structure more or less enclosed
between the two auricles and from which the bulbus cordis
arises anteriorly (Pl. 5, figs. 1 and 2).   The ventral surface
of the ventricle presents no remarkable features.   Near the
apex there is the little fibrous band (Pl. 5, fig. 7) that binds the
heart to the pericardium (this has been dissected away in Pl.
5, figs. 1 and 2).   The continuity of the dorsal wall of the
ventricles is interrupted by the large horse-shoe-shaped auri-
culo-ventricular opening that lies in the middle of the dorsal
surface of the heart (Pl. 5, figs. 1 and 2).   The margins of this
aperture sweep round on either side to meet the conspicuous
auriculo-ventricular plug posteriorly.   Externally there is no
indication of any division of the ventricular part of the heart
into two compartments.

Attached, and immediately anterior to the posterior margin
of the auriculo-ventricular opening, lying over the aperture
—really in it—is a prominent, rather button-like structure of
cartilaginous consistency—the auriculo-ventricular plug (Pl.
5, figs. 1 and 2, *A. V. pl.*).   This structure, being in front of
the posterior margin of the auriculo-ventricular opening, is
enclosed by the auricular walls, and, owing to the posterior

part of the interauricular septum (pulmonary fold, Pl. 5, fig. 1, *p. f.*) being attached across the middle of its dorsal surface, the left half of that surface is enclosed in the left, the right in the right auricle; its ventral surface gives attachment to the interventricular septum (Pl. 5, figs. 1 and 2, *A. V. pl.* and *V. S.*). This plug, as stated, lies over the auriculo-ventricular aperture, and when approximated against it closes it accurately.

The interventricular septum is a thick muscular partition, whose fibres radiate fanwise from the ventral surface of the auriculo-ventricular plug to the apex and ventral and lateral walls of the ventricle, its free anterior border (round which the ventricles can communicate) passes posterior to the ventriculo-bulbar opening (Pl. 5, figs. 1 and 2, *V. S.*).

Bulbus Cordis.—The bulbus cordis is a tubular structure that arises anteriorly from the dorsal surface of the ventricles and forms a considerable portion of the heart. It presents a characteristic transverse bulging in its middle part, and its dorsal and lateral surfaces are partially concealed by the auricles; reference has already been made to its lymphatic sheathing.

The bulbus may be divided into three parts: (1) A comparatively short proximal part directed antero-posteriorly and opening from the ventricles a little anterior to the free margin of the interventricular septum (Pl. 5, figs. 1, 2 and 4, *B. C. p.*); (2) a short transverse part (demarcated externally from (1) by a distinct circular groove), directed from right to left and exhibiting a marked bulging of its ventral wall (Pl. 5, figs. 1, 2 and 4, *B. C. t.*); (3) a longer distal part, again directed antero-posteriorly (Pl. 5, figs. 1, 2 and 4, *B. C. d.*), and whose apex forms the extremely short ventral aorta from which the aortic arches take origin (see Pl. 5, fig. 4, *S. Ao.*). The transverse part of the bulbus cordis forms a distinct characteristic prominence on the ventral surface of the heart; the proximal part has a well-developed circular musculature, while the muscular coat of the transverse and distal parts is poorly developed.

In the proximal portion of the bulbus there is, attached to the ventral wall, a solid ridge which extends to the transverse part (Pl. 5, figs. 1 and 3, *Sp. V. p.*) ; this ridge or valve is inserted along the middle line of the ventral bulbus wall, commencing at its posterior end a little anterior to, but immediately in line with, the interventricular septum (Pl. 5, figs. 1 and 2, *V. S.*, and *Sp. V. p.*) ; proximally it tapers off rapidly into the bulbus wall, but distally it broadens considerably, while its somewhat flattened end projects forwards into the lumen of the transverse part of the bulbus (Pl. 5, figs. 3 and 4, *S p. V. p.*). On this valve near its origin there is always one distinct transverse ridge, in front of which again one or two more or less faint transverse furrows are usually to be distinguished (Pl. 5, figs. 1 and 3, *t. f.*) ; these recall the superimposed valves from which the spiral ridge is believed to have been evolved in phylogeny. At the same level, traces of vestigial pocket valves (**17**) are present on the lateral and dorsal walls of the bulbus cordis ; these are represented usually by three rows of tiny ridges arranged three in each row with still tinier irregular vestiges between (Pl. 5, figs. 1 and 3, *a, b, c*). In one case, out of eight hearts examined, only two vestigial valves could be distinguished in each row. The proximal ridges, those nearest the ventricle, are always most prominent.

As the transverse part of the bulbus is reached, the valvular ridge on the ventral wall of the first part is continuous with the ledge projecting from the dorsal wall of the transverse part (Pl. 5, figs. 1 and 4, *Sp. V. t.*). This vertical ridge in the transverse part is usually distinctly concave on its posterior surface, but in one instance it was characterised by the dorso-ventral flattening of its free ventral margin so as to give it in consequence a somewhat ⊥-shape. In the distal part of the bulbus the valve becomes more flattened, has a free left border, and is attached along the right wall (Pl. 5, figs. 2 and 4, *Sp. V. d.*) ; these continuous valve-like structures curving along the length of the bulbus cordis constitute the spiral valve. In the distal part of the bulbus there is also a second longitudinal valve-like projection

attached along the left wall somewhat dorsally (Pl. 5, figs. 2 and 4, *B. R.* 3.); this unites for a short distance at its distal end with the spiral valve, dividing the cavity of the ventral aorta into a dorsal and ventral passage; this short partition wall fuses at its distal extremity with the dorsal wall of the ventral aorta in front of the region of the fifth and sixth aortic arches, terminating in a little cushion-like projection (Pl. 5, figs. 2 and 4, *S. Ao.*). The result is the formation of a ventral passage communicating with the two anterior, and a dorsal passage communicating with the two posterior, pairs of aortic arches.

Coronary Arteries.—Two little arteries are present one on each side of the bulbus cordis. The right vessel is the larger; it passes along the right wall of the distal part of the bulbus, dorsal to the transverse part, and then along the left wall of the proximal part, to the ventricle, where it is distributed. The smaller left artery supplies the left dorsal wall of the distal part of the bulbus. The course of these little vessels anterior to their appearance on the sides of the bulbus has not been traced, owing to the difficulty of dissecting the fibrous tissue in the region of the ventral aorta.

Summary.—The main features of importance in the anatomy of the heart of Lepidosiren may now be summarised, and some suggestions put forward as to their probable physiological significance.

In Lepidosiren, as we have seen, auricle, ventricle and bulbus cordis are each more or less incompletely divided into two right and left chambers, and as the septa of all these compartments (the proximal part only of the bulbus being considered for the moment) are approximately in the same plane, it follows, therefore, that the three chambers on the right and the three on the left are in sequence respectively. The sinus venosus opens into the right auricle, and its aperture is protected on the right side by a more or less efficient valve (Pl. 5, fig. 1, *r. S. A.*). The pulmonary vein opens directly into the left auricle and is also protected by a

valve-like fold (Pl. 5, fig. 2, *P. f.*). The auricles open into the ventricles round the margins of the auriculo-ventricular plug and there are no auriculo-ventricular valves apart from this (Pl. 5, figs. 1 and 2, *A. V. pl.*); the ventricular opening is guarded and closed from the side of the ventricle by the auriculo-ventricular plug; the bulbus cordis opens directly from the ventricle, and its aperture is undefended by any but the vestigial valves previously described (Pl. 5, figs. 1 and 3, *a, b, c*).

In the absence of direct physiological observations we may assume that the heart functions are as follows: venous blood from the sinus venosus and arterial blood from the pulmonary vein enters the right and left auricles respectively; when the auricles contract the sinu-auricular opening will be closed, partly by the action of the valve guarding its right side and partly by the bulging of the slack right wall of the pulmonary vein into it (Pl. 5, fig. 1, *P. f.*), while regurgitation along the pulmonary vein will be prevented partly by the action of the margins of the pulmonary aperture itself and partly by the marked obliquity of the pulmonary vein immediately before entering the auricular cavity (Pl. 5, fig. 2, *P. V.*); the contents of the auricles will then be discharged into the ventricles on either side of the auriculo-ventricular plug. With the contraction of the ventricles and of the interventricular septum the auriculo-ventricular plug is drawn in a ventral direction, and this, with the contraction of the muscles encircling it, closes the auriculo-ventricular aperture; simultaneously the proximal end of the bulbus is approximated to the interventricular septum, and the contents of either ventricle are discharged along the corresponding side of the spiral valve of the bulbus. On the contraction of the proximal muscular part of the bulbus cordis the two blood-streams are guided on either side of the septum of the first part to the transverse portion; in the transverse part of the bulbus the blood from the right side of the heart passes along the posterior channel behind the vertical septum, while the blood from the left side of the heart passes along the anterior

channel; this relationship is maintained in the third part of
the bulbus where the venous stream passes dorsal to the two
septa and so enters the posterior pair of aortic arches, while
the arterial stream passes ventral to the septa and enters the
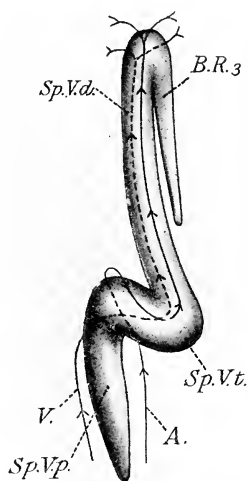anterior aortic arches (Text-fig. 1 and Pl. 5, fig. 4).

TEXT-FIG 1.

Diagram of valves in the bulbus cordis as seen from the ventral
side. The line A indicates the course of the arterial blood, the
line v that of the venous blood. *Sp. V.p.* Spiral valve on the
ventral wall of the proximal part of the bulbus. *Sp. V. t.* Spiral
valve on the dorsal wall of the transverse part of the bulbus.
Sp. V. d. Spiral valve on the right wall of the distal part of the
bulbus. *B. R. 3.* Longitudinal valve on the left wall of the
distal part of the bulbus.

Owing to the very small amount of musculature in the walls
of the middle and distal segments of the bulbus, the propulsive
movement in those parts must be more an elastic recoil than
an active contraction.

Regurgitation from bulbus to ventricle will be prevented
partly by the plugging effect that the distal end of the
prominent valve in the proximal part must have on a back-

wardly directed current (Pl. 5, figs. 1, 3 and 4, *Sp. V. t.*).
Again at the distal end of the bulbus the anterior termination
of the aortic septum does not taper off imperceptibly into the
dorsal wall, but presents a solid vertical surface that would
offer some resistance to regurgitation from the two anterior
pairs of aortic arches (Pl. 5, figs. 2 and 4, *S. Ao.*).

It is of interest to note that the grooves formed between
parts (1) and (2) and parts (2) and (3) of the bulbus are
respectively homologous with the proximal and distal "Knick-
ungsfurche" described by Greil in the developing bulbus
cordis of Lacerta (**9**).

B. Arteries.

In the adult four afferent vessels arise in two sets of two in
close proximity on either side, from the very short ventral
aorta at the anterior end of the bulbus cordis (Pl. 5, fig. 1,
A. A.).

Dorsally four efferent vessels join on either side to form the
dorsal aortic roots, and these again, by their junction in
the middle line, form the dorsal aorta (Text-fig. 2 *Ao.*) ;
the two posterior pairs of afferent vessels arise somewhat
from the dorsal surface of the ventral aorta, while the two
anterior pairs are placed more ventrally. The proximal part
of the anterior vessel on either side is really the paired ventral
aorta, which, after passing a short distance outwards and
forwards in the floor of the mouth, is prolonged into the
lingual artery (Text-fig. 2, *L. A.*) anteriorly, immediately
posterior and external to which it gives off the third aortic
arch (the most anterior of the persisting aortic arches). In
the adult this aortic arch (Text-fig. 2, *A. A.* 3) curves outwards
dorsally and backwards and then inwards to the outer margin
of the roof of the mouth, where it joins the dorsal aortic root
at the point of origin of the dorsal carotid artery (Text-fig.
2, *Car.*). The dorsal root then passes inwards and slightly
backwards, and is joined in rapid succession, a short distance
from the middle line, first by the fourth, and then by the short
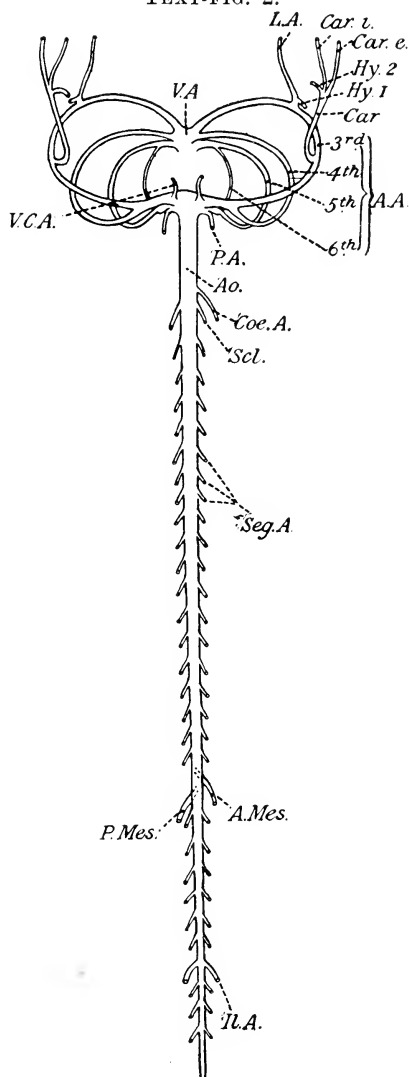
TEXT-FIG. 2.

Diagram of arterial system, dorsal view. *AA.* 3, 4, 5, 6. The four
aortic arches. *A. mes.* Anterior mesenteric artery. *Ao.* Aorta.
Car. Dorsal carotid. *Car. e.* External branch of carotid. *Car. i.*
Internal branch of carotid. *Coe. a.* Cœliac artery. *Hy.* 1.
Branch to hyoidean hemibranch. *Hy.* 2. Hyoidean efferent
artery. *Il. A.* Iliac artery. *L. A.* Lingual artery. *P. A.* Pul-
monary artery. *V. C. A.* Vertebro-cerebral artery. *P. mes.*
Posterior mesenteric artery. *Scl.* Subclavian artery. *Seg. A.*
Segmental arteries. *V. A.* Ventral aorta.

common stem of the fifth and sixth aortic arches. In the middle line the dorsal aortic roots meet and form the dorsal aorta (Text-fig. 2, *Ao.*). The fourth pair of aortic arches takes origin from the proximal part of the lateral ventral aorta on either side and curves outwards, upwards and inwards to its junction with the dorsal aortic root. The fifth and sixth pairs arise by short common stems from the proximal part of the ventral aorta. From these common stems the sixth aortic arches, which are short and of extremely small calibre, are given off almost immediately. The fifth and sixth aortic arches on either side curve round the postero-lateral regions of the mouth-cavity, and rejoin one another dorsally, entering the dorsal aortic roots near the middle line by a short common stem. The small size of the vessels of the sixth aortic arches is correlated with the fact that in the adult Lepidosiren the pulmonary arteries no longer arise directly from them as they did in the larva, but from the common stems of the fifth and sixth arches on either side, and apparently the fifth arch has taken on the larger share of the blood supply, while the sixth arch has dwindled to a very insignificant vessel. The channel by which arches 5 and 6 communicate with the dorsal aorta is relatively small compared with the lumen of the pulmonary artery, the main blood-stream passing into the latter vessel. In Lepidosiren all the aortic arches can be traced throughout as definite uninterrupted vessels.

Vertebro-cerebral Arteries.—Immediately before the dorsal aortic roots join to form the dorsal aorta, two little vessels, the vertebro-cerebral arteries, pass from them one on either side to the base of the skull (Text-fig. 2, *V.C.A.*).

Dorsal Aorta.—The dorsal aorta (Text-fig. 2, *Ao.*) is formed by the junction of the dorsal aortic roots far forwards about the level of the junction of the distal and transverse portions of the bulbus cordis. It extends throughout the length of the spinal column immediately ventral to it, and in the caudal region lies in the haemal canal, dorsal to the caudal vein. The main vessels arising from the dorsal aorta are the cœliac, subclavian, anterior and posterior

mesenteric and the iliac arteries (Text-fig. 2, *Coe. A., Scl., A. mes., P. mes.,* and *Il. A.*). Of these the limb vessels are paired, the others unpaired. Posterior to the subclavian arteries the aorta gives off segmentally arranged vessels (Text-fig. 2, *Seg. A.*), which in turn give branches to the spinal column, body-walls, and to the kidney and gonad.

Lingual Arteries.—On either side the paired ventral aorta is prolonged forwards as the lingual artery (Text-fig. 2, *L. A.*); this vessel passes outwards and forwards along the floor of the mouth between the ramus of the jaw, to which it gives a branch, and the tongue.

Carotid Arteries.—The dorsal aortic root on either side is prolonged forwards as the dorsal carotid artery (Text-fig. 2, *Car.*); this vessel passes forwards a short distance in the roof of the mouth, as far as the anterior part of the auditory capsule; here it divides into an internal and an external branch (Text-fig. 2, *Car. i., Car. e.*). The external branch passes forwards slightly dorsally and outwards, accompanying the trigeminal nerve, with which it is distributed to the surface of the head. The internal branch passes dorsally a little, to the base of the brain itself.

Pulmonary Arteries.—Two pulmonary arteries (Text-fig. 2, *P. A.*) arise one on either side from the short common dorsal stem of the fifth and sixth aortic arches; both vessels extend for some distance dorsal to the œsophagus on either side of the aorta, but as they reach the lungs their positions relative to one another alter. Of the two vessels the left artery is the larger; it passes backwards and inwards and curves from the left side round the ventral surface of the œsophagus to reach the ventral surface of the lungs, and then, after crossing ventrally to the left pulmonary vein, bifurcates, giving a branch to each lung. The right pulmonary artery is the smaller: on reaching the lungs it passes backwards and inwards, to the left of and ventrally to the cœliac artery, and divides into two branches, one to the dorsal surface of each lung.

Cœliac Artery.—The cœliac artery (Text-fig. 2, *Cœ. A.*)

arises from the dorsal aorta on the right side a short distance behind the point of junction of the two aortic roots. It passes first ventrally, then posteriorly, along the right outer angle of the liver, and crosses the ventral surface of that organ to reach the tip of the gall-bladder, dorsal to which it finally reaches the intestine, in the wall of which it is distributed.

Subclavian Arteries.—Two small vessels, the subclavian arteries (Text-fig. 2, *Scl.*), arise one on either side of the aorta immediately posterior to the cœliac artery and pass outwards to supply the pectoral limbs.

Anterior Mesenteric Artery.—This vessel (Text-fig. 2, *A. mes.*) arises from the mesial ventral surface of the dorsal aorta far back, a short distance in front of the iliac arteries, and passes in the mesentery to reach the walls of the intestine, to which it is distributed.

Posterior Mesenteric Artery.—Immediately posterior to the anterior mesenteric artery a second vessel, the posterior mesenteric (Text-fig. 2, *P. mes.*), arises also from the mesial ventral surface of the dorsal aorta and passes through the mesentery to the intestine, to which it also is distributed.

Iliac Arteries.—Posteriorly two small vessels (Text-fig. 2, *Il. A.*) arise from the dorsal aorta near its caudal extremity and are distributed, one on either side to the pelvic limbs.

c. Veins.

Ductus Cuvieri.—A short transverse ductus Cuvieri opens into the anterior part of the sinus venosus (Text-fig. 3, *S. V.*) on either side ; these vessels are formed by the junction of the anterior and posterior cardinal veins (Text-fig. 3, *r. D. C.* and *l. D. C.*).

Anterior Cardinal Veins.—On each side an anterior cardinal vein (Text-fig. 3, *A. Car.*) passes back superficially from the front of the upper jaw, receiving an anterior cerebral vein in front of, and an orbital vein immediately behind, the eye, beneath which organ it passes : it also receives a vessel from the surface of the lower jaw at the angle of the mouth. Posteriorly a venous trunk, the posterior cerebral vein (Text-
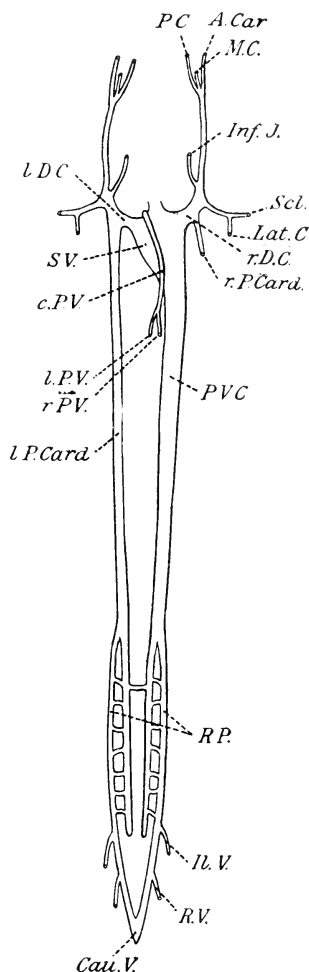
TEXT-FIG. 3.

Diagram of venous system, dorsal view. *A. Car.* Anterior cardinal. *Cau. v.* Caudal vein. *c. P. V.* Common pulmonary vein. *Il. V.* Iliac vein. *Inf. J.* Inferior jugular. *Lat. C.* Lateral cutaneous. *l D C.* Left duct of Cuvier. *l.P.V.* Left pulmonary vein. *l. P. Card.* Left posterior cardinal. *M. C.* Vestige of median cephalic vein. *P.C.* Posterior cerebral vein. *P. V. C.* Posterior vena cava. *r. D. C.* Right duct of Cuvier. *R. P.* Renal portal vein. *r. P. Card.* Right posterior cardinal. *r. P. V.* Right pulmonary vein. *R. V.* Rectal vein. *Scl.* Subclavian. *S. V.* Sinus venosus.

fig. 3, *P. C.*), passes backwards and outwards from the inside of the skull, and then turns sharply in a ventral direction to join the anterior cardinal at the lower part of, and posterior to, the auditory capsules. At the junction of the cerebral vein with the anterior cardinal they are joined by a third very wide short vessel (Text-fig. 3, *M. C.*), passing between the skull and the surrounding musculature, which is apparently a vestige of the median cephalic vein present in the embryo. From this point the cardinal vein passes ventrally and a little inwards, curving backwards, dorsal to the aortic arches, to be joined on the dorsal surface of the pericardium by the subclavian and inferior jugular veins (Text-fig. 3, *Inf. J.* and *Scl.*) before joining the posterior cardinal vein (Text-fig. 3, *l.* and *r. P. Card.*) to form the duct of Cuvier (Text-fig. 3, *r. D. C.* and *l. D. C.*).

Inferior Jugular Veins.—On either side of the floor of the mouth an inferior jugular vein (Text-fig. 3, *Inf. J.*) passes backwards below the afferent branchial vessels along the roof of the pericardium, and joins the respective anterior cardinal on its inner ventral surface near the anterior end of the ductus Cuvieri.

Subclavian Veins.— From each pectoral limb a vein passes inwards and forwards to join the anterior cardinal vein near the junction of that vessel with the inferior jugular vein (Text-fig. 3, *Scl.* and *Inf. J.*).

Caudal and Renal Portal Veins.—A caudal vein (Text-fig. 3, *Cau. V.*) runs beneath the caudal aorta enclosed in the hæmal canal. On emerging from this it divides to form the paired renal portal veins (Text-fig. 3, *R. P.*), which pass along the outer ventral margins of the kidneys to terminate at their anterior ends and anastomose through the substance of the kidney with the left posterior cardinal and the posterior vena cava (right posterior cardinal) respectively (Text-fig. 3, *l. P., Card.* and *P. V. C.*).

Iliac Veins.—Two iliac veins from the pelvic limbs enter the renal portal veins, one on either side, shortly after these enter their respective kidneys (Text-fig. 3, *Il. V.*).

Left Posterior Cardinal Vein and Posterior Vena Cava.—The left posterior cardinal vein and the posterior vena cava (right posterior cardinal) appear on the dorso-mesial surfaces of the posterior part of the left and right kidneys respectively; they communicate through these organs by an intricate meshwork of venous capillaries with the left and right renal portal veins (Text-fig. 3, *l. P. Card.* and *P. V. C.*).

The left posterior cardinal vein, on leaving the kidney, passes forwards between the intestine and the body-wall ventrally to the left lung, and passing along the left of the roof of the sinus venosus, joins the posterior termination of the left anterior cardinal vein to form the left duct of Cuvier (Text-fig. 3, *l. D. C.*).

The posterior vena cava (right posterior cardinal vein) passes forwards from the right kidney, and inclining somewhat to the middle line, is embedded in the dorsal surface of the liver; here it receives a number of venous radicles from the liver substance. Immediately on emerging from the anterior end of the liver it enters the sinus venosus (Text-fig. 3, *P. V. C.*). In the one adult injected Lepidosiren in which this part was dissected, there was only one large transverse anastomosis towards the posterior part of the liver, between the left posterior cardinal vein and that part of its fellow on the right side that forms the hind part of the posterior vena cava (13). Hyrtl mentions four such anastomoses in an adult specimen.

The renal portal veins posteriorly and the left posterior cardinal and posterior vena cava anteriorly receive veins from the body-walls and also vertebral veins. These are segmentally arranged.

Right Posterior Cardinal Vein.—The anterior portion of the right posterior cardinal vein (Text-fig. 3, *r. P. Card.*) is present as a short vessel near the posterior end of the heart; it receives a vein from the region of the vertebral column and one from the body-wall, and the trunk so formed joins the posterior part of the right anterior cardinal vein to form the right duct of Cuvier. A small vertebral vessel joins the left posterior cardinal vein also in this region.

Pulmonary Veins.—Posteriorly the pulmonary veins lie along the outer borders of the lungs, but turn inwards at about the anterior third of those organs across their ventral surfaces, and join to form the common pulmonary vein (Text-fig. 3, c. P. V.) somewhat to the right of the middle line. The common pulmonary vein passes forwards and to the left on the dorsal wall of the anterior portion of the posterior vena cava, and entering the pericardium reaches the roof of the sinus venosus. It then runs obliquely to the left in the roof of the sinus venosus and curves ventrally across the anterior end of the sinus, to open into the left auricle on the left side of a fold that projects into the auricles, across the middle of the dorsal surface of the auriculo-ventricular plug (Pl. 5, fig. 1, P.f.). This pulmonary fold is continuous dorsally with the auricular roof, anteriorly with the muscular part of the auricular septum, and ventrally with the auriculo-ventricular plug. The pulmonary aperture is a rounded opening in the left wall of this pulmonary fold, and is guarded by a hood-like flap that serves to guide the blood directly to the left on entering the auricle (Pl. 5, fig. 2, P. V. and P. f.).

Coronary Vein.—A small coronary vein passes from about the middle of the dorsal surface of the right ventricle to reach the floor of the sinus venosus a short distance from the sinu-auricular aperture; its opening is guarded by a valvular flap whose free border is directed posteriorly. The exact point at which this little vessel passes from the ventricle to the sinus varies considerably in different specimens (Plate 5, fig. 1, C. V.).

III. Development of the Heart.

Pericardiac Space.—At Stage 23, in the development of Lepidosiren, when there are twenty-four segments present, the lateral mesoderm is extending ventrally and inwards over the surface of the yolk on either side of the endodermic pharyngeal rudiment, and already two chink-like cœlomic

splits are present, one on either side, between its layers
(Text-fig. 4, *co.*).

These two folds of mesoderm, with their contained chinks,
soon extend across the surface of the yolk, and meeting in
the middle line, ventral to the pharyngeal rudiment, fuse to
form the pericardiac portion of the cœlom (Stage 24, Text-
fig. 5 A, B and C, *spl. m.* and *som. m.*).

Owing to the spherical shape of the yolk and to the close

TEXT-FIG. 4.[1]
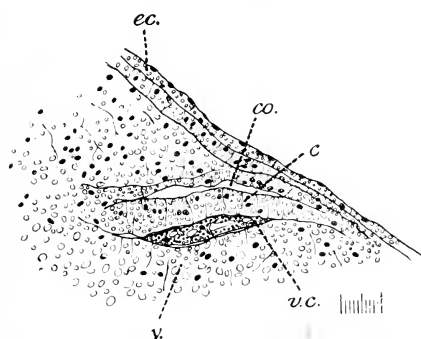
Transverse section, Stage 23. Cœlom just appearing, columnar
splanchnic mesoderm defined, vessel cells present. *c.* Columnar
layer of mesoderm. *co.* Cœlom. *ec.* Ectoderm. *v. c.* Vessel
cells. *y.* Yolk.

approximation of the embryo round it, the pericardiac cœlom
is, from the first, flattened, while it is curved transversely
round the anterior surface of the yolk, and also dorsally a
little on either side of the pharyngeal rudiment. Thus this
part of the cœlom is somewhat crescent-shaped, with the
pharyngeal endoderm resting between its horns, and also
a little concave posteriorly owing to the curvature of the
yolk. The walls of the pericardiac cavity consist of a single
layer of cells, of which those of the splanchnic wall are
distinctly columnar in shape, giving rise later to the myo-
cardium, while those of the somatic wall are comparatively
flattened cells from which the parietal pericardium is derived.

[1] Each division of the scale in this and succeeding figures represents
·01 mm.

Rudiments of the Vitelline Veins, Heart and Aorta.—Simultaneously, with the progressive delamination of the anterior margins of the lateral mesoderm from the surface of the yolk, two slender irregular strands of cells (Text-fig. 4, *v. c.*) appear, one on either side, between the yolk and the splanchnic layer of mesoderm. These are em-
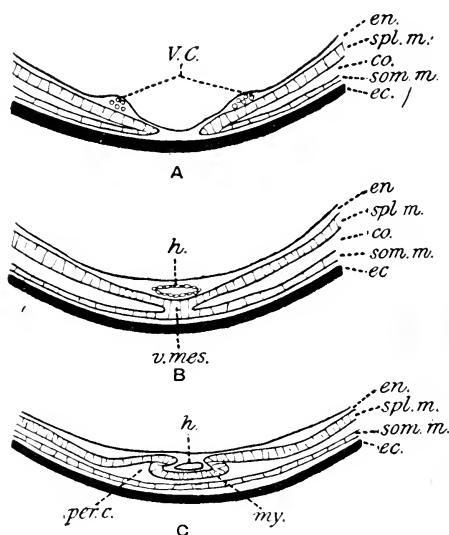
TEXT-FIG. 5.

Diagram illustrating the relations of the heart-rudiment to the mesoderm layers and cœlom. *co.* Cœlom. *ec.* Ectoderm. *en.* Endoderm yolk. *h.* Heart. *my.* Myocardium. *per. c.* Pericardiac cœlom. *som. m.* Somatic mesoderm. *spl. m.* Splanchnic mesoderm. *V. C.* Vessel cells. *v. mes.* Ventral mesocardium.

bryonic endothelial cells, and they extend inwards across the anterior surface of the yolk simultaneously with the development of the mesoderm and cœlom. These cells are comparatively large and heavily yolked, and except peripherally, where they are continuous with the inner mesoderm layers over the lateral surfaces of the yolk, they are quite distinct from the endoderm and mesoderm, between which they lie.

With the fusion of the mesoderm below the ventral surface of the pharynx and the formation of the bilateral

TEXT-FIG. 6.
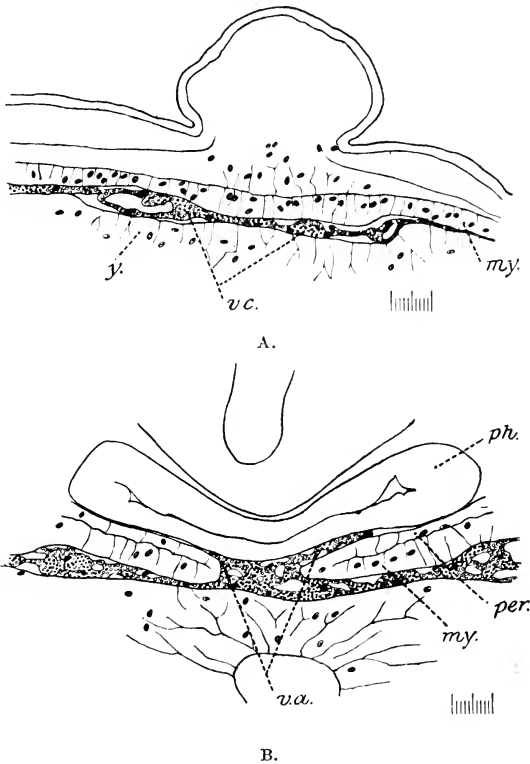
A. Section transverse to the heart-rudiment at Stage 24. B. Section transverse to the heart, showing endothelial rudiments of the paired ventral aortæ. *my.* Myocardium. *per.* Outer wall of pericardiac cavity. *ph.* Pharyngeal rudiment. *v. a.* Rudiment of the paired ventral aortæ. *v. c.* Vessel-cells with unexpanded cardiac cells between them. *y.* Yolk.

pericardiac cœlom, the two endothelial strands meet on the anterior surface of the yolk, and form a little single-layered sheet of endothelial cells forming the rudiment of the heart (Stage 24, Text-fig. 6 A, *v. c.*).

This fusion of the mesoderm below the pharynx occurs

over a very short space, and the heart-rudiment is corre-
spondingly short also. A trifle later (Stage 24 +), a strand
of embryonic endothelial cells is found, continuous with and
anterior to the heart-rudiment immediately ventral to the
pharyngeal rudiment, and from it again two similar strands
pass, one on either side, sharply outwards and a little back-
wards and dorsally to the sides of the head between the
parietal pericardium and the lateral expansions of the pharyn-
geal rudiment: these prolongations are the rudiments of
the lateral ventral aortæ (Text-fig. 6b, *v. a.*). Intra-cellular

TEXT-FIG. 7.
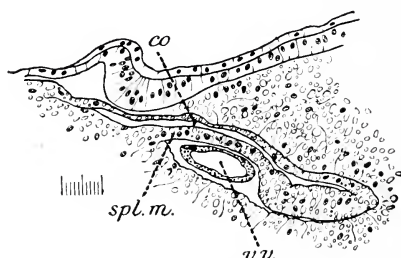
Expanding vitelline vessel and splanchnic layer of mesoderm
bulging forwards into the cœlom. *co.* Cœlome. *spl. m.* Splanch-
nic mesoderm. *v. v.* Vitelline vessel.

spaces, due to metabolic processes accompanied by the
secretion of fluid, soon form in these various embryonic cells
in the order of their appearance, that is to say, first in the
vitelline veins, then in the heart, and finally in the rudiments
of the ventral aortæ (Text-figs. 4, 6a and b, *v. c.* and *v. a.*).
These spaces increase in size, adjacent cells coalesce, and finally
definite endothelial vessel-tubes result (Text-fig. 7, *v. v.*).

Simultaneously with the expansion of the endothelial
tubes the splanchnic mesoderm bulges before them into the
cœlomic cavities and affords a covering to the developing
vessels (Text-fig. 7, *spl m.*). As the middle cells of the heart-
rudiment vacuolate and expand a trifle later than those at its
lateral margins (Text-fig. 6a, *v.c.*), it has for a while a
somewhat dumb-bell-like appearance on horizontal section,

but this condition is transitory, though the flattened oval shape of the lumen of the heart-tube—attributable probably to the spherical surface of the yolk and the close approximation of the embryo against it—persists for a considerable time.

This characteristic arrangement of the rudiments of the vitelline veins, heart and ventral aortæ is probably due to the presence of the yolk. Instead of being extended in the long axis of the embryo with the rudiments of the great vessels appearing far apart at either end of it, the heart-rudiment has suffered an approximation of its cranial and caudal extremities, while the intervening part has become folded on itself and projects ventrally over the yolk. This arrangement, therefore, is merely an expression of the relations of embryo and yolk peculiar to Lepidosiren.

Shape and Attachments of the Heart.—From the first the heart-rudiment is much flattened between the head of the embryo and the yolk, and the comparative approximation of its two ends is maintained throughout development. This, as already suggested, is determined by the presence of the yolk and by the flattened, laterally expanded shape of the pericardiac space, both of which conditions, of course, are interdependent and persist for a comparatively long time. These factors also account for the vertical position of the heart, relative to the long axis of the embryo, during its earlier stages of development.

When the mesoderm plates meet ventrally in the cardiac region they fuse rapidly, and the extremely short ventral mesocardium disappears almost as soon as formed (Stage 24 +, Text-fig. 5B, v. mes.). The heart now grows rapidly in length, and as its anterior and posterior ends remain relatively fixed it projects ventrally more and more into the narrow pericardiac space and forms a loop, flattened antero-posteriorly (Text-fig. 8). This rapid growth of the heart-tube, combined with the peculiar shape of the cavity in which it is placed, causes a degree of twisting, the immediate effect of which is to free the loop from the dorsal mesocardium, so that now the heart has a complete myocardiac covering, only the dorsal

wall of the developing sinus venosus being still in close
relationship with the pharyngeal rudiment above.   In this
adjustment of the heart-tube to the pericardiac space, the
descending and ascending limbs of the loop, from being pos-
terior and anterior respectively, come to lie side by side, the
former on the left and the latter on the right (Text-fig. 8).
That is to say, the anterior and posterior ends of the heart are
fixed, but the loop as a whole rotates approximately through
a right angle in a clockwise direction as seen in a dorsal
view of the heart, thus twisting the long axis of that organ
into a position approximately transverse to, instead of parallel
with, the axis of the embryo.   This position is maintained
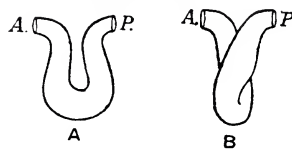
TEXT-FIG. 8.

Diagram to illustrate the looping of the heart on itself ; (A) repre-
    sents the primitive and (B) the secondary position of the heart.
    A. Anterior.   *P*. Posterior.

till the maximum vertical development of the heart has been
attained, when a degree of untwisting occurs as the pericar-
diac space begins to increase rapidly in antero-posterior
depth, and the adult form and position of the heart are
reached.

    From Stage 25 the heart consists for a time of a double-
walled tube, the inner wall being endothelial and the outer
myocardial (Text-fig. 9).

    The tube is bent in a narrow U-shape, its anterior and
posterior ends being in close proximity to one another, and
both approximately in the middle line, the former terminating
on either side in the lateral ventral aorta, the latter forming
the sinus venosus, somewhat to the right.   This double U-tube
may be divided into four parts : (1) A posterior descending
auricular part (Text-fig. 9 A, *A*.), the axis of which forms

TEXT-FIG. 9.
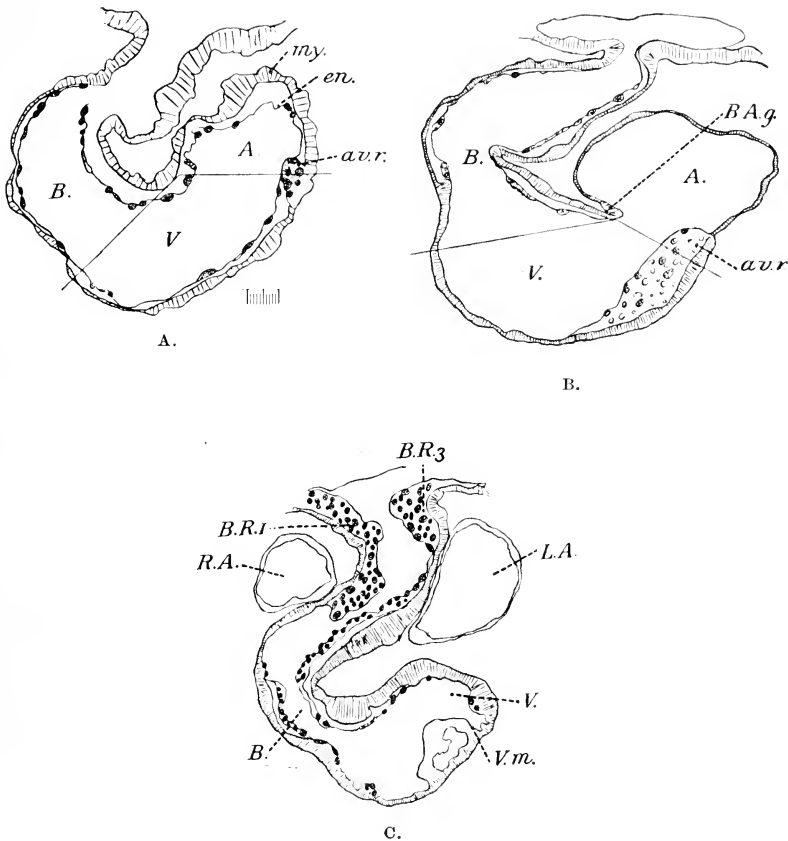
Sections transverse to the body of the embryo, illustrating the
development of the heart. A. Stage 28. Heart markedly **U**-
shaped, endothelium and myocardium still separate. The
straight lines demarcate approximately the different divisions
of the heart. B. Stage 30. C. Stage 31. *A.* Auricle. *av. r.*
Auriculo-ventricular ridge, which in fig. B extends to the ventral
curvature of the ventricular part of the heart. *B.* Bulbus.
B. Ag. Bulbo-auricular groove. *B. R.* 1. Right bulbus ridge.
B. R. 3. Left bulbus ridge. *en.* Endothelium. *L. A.* Left
auricle. *my.* Myocardium. *R. A.* Right auricle. *V.* Ventricle.
V. M. Ventricular musculature continuous posteriorly with the
auriculo-ventricular ridge.

almost a right angle with the sinus venosus and projects
to the left into the pericardiac cavity; (2) a short auricular
canal intervening between (1) and (3); (3) a ventral, trans-
verse, ventricular part (Text-fig. 9 A, *V*.) directed from left
to right, and (4) an anterior, ascending part, curving from
right to left towards the middle line again—the forerunner
of the bulbus cordis (Text-fig. 9 B, *B*). The long ventral
and short dorsal walls of the transverse part of the tube
may now, for brevity, be called the ventral and dorsal
curvatures of the heart respectively.

Auriculo-ventricular Plug.—At Stage 27, before
there is any definite division of the heart into separate

TEXT-FIG. 10.
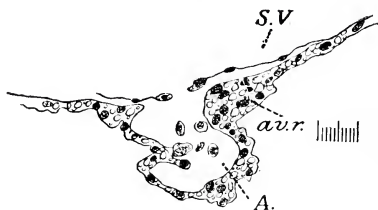
Section through the sinu-auricular junction at Stage 28. *A.* Auricle.
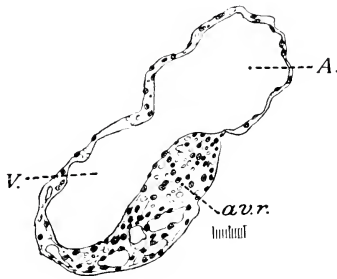*av. r.* Auriculo-ventricular ridge. *S. V.* Sinus venosus.

chambers, a little knot of cells appears between the two
layers of the heart-wall, at the left ventral margin of the
opening of the sinus venosus into the auricle (Text-fig. 10,
av.r.).

This knot of cells forms a ridge extending in a ventral
direction along the left posterior wall of the heart through
the auricular canal, and reaching as far as the commencement
of the ventral curvature (Text-figs. 9 A and B, *av. r.*) On the
ventral curvature the tip of the ridge comes in contact and
becomes continuous with the muscle-fibres appearing in the
ventricle (Text-fig. 11, *av. r.*). This ridge inclines from left
to right (compare fig. 15A, B, and C, *av. r.*) and divides the
auricle into a larger right and a smaller left compartment,
the former being situated at first somewhat posterior to the

latter. The sinu-auricular aperture opens on the right of the ridge and is therefore confined to the right auricle. Throughout, the right auricle remains the larger, though the left expands rapidly—as does also the left ventricle—after the development of the pulmonary vein.

Division of the Heart into Chambers.—As development proceeds the various chambers of the heart come to be demarcated from one another; this is largely brought about by a marked disproportion in the rates of growth at different parts. For a time growth occurs chiefly in

TEXT-FIG. 11.

Sagittal section through the heart at Stage 30. *A.* Auricle. *av. r.* Auriculo-ventricular ridge becoming continuous with muscular tissue in the ventricle. *V.* Ventricle.

length and width; a little later, however, the pericardiac cavity increases in anteroposterior depth, more especially in its ventral part, and this allows the heart, as already mentioned, to regain a position approximately parallel with, instead of transverse to, the long axis of the embryo. In fact from this stage till the permanent condition is attained—as the liver develops and retreats somewhat towards the tail, as the left auricle and ventricle expand with the appearance of the pulmonary vein, as the yolk is absorbed and the adult shape of the anterior part of the body is reached—a certain rotation of the loop takes place. The heart loop gradually swings back again in a counter-clockwise direction, as seen in a dorsal view, about an axis perpendicular to the long

axis of the embryo, till, from being practically at right angles
to, the heart loop is once more parallel with, the length of
the embryo (Pl. 5, figs.5, 6 and 7). Throughout it must be
remembered that the anterior and posterior ends of the heart,
are fixed, and therefore whatever rotation occurs affects only
the loop of the tube, and further, that the second rotation
is merely a recovering of the original position of the primitive
endothelial rudiment before its peculiar environmental rela-
tions compelled it to adopt a transverse position. Ultimately,
therefore, the amount of twisting displayed by the heart as a
whole is negligible. Also as the yolk disappears, the heart,
from being vertical (Text-figs. 9 and 12), becomes more and
more horizontal in position, the ventricular axis coming to
form a comparatively acute angle with the common axis of
sinus and auricle (Pl. 5, fig. 1). Thus in Lepidosiren,
owing to the exigencies of the yolk, the definitive position of
the heart is not finally assumed till a fairly late stage in
development is reached, which renders a description of its
changing anatomical relations peculiarily difficult. In the
meantime, however, for the sake of simplicity, the heart will
be considered as vertical to the long axis of the embryo, the
position which is maintained more or less till development is
practically complete, and with its loop rotated into the defi-
nitive antero-posterior position. The terms "anterior" and
" posterior" thus express in the earlier stages relationships
that in the adult are ventral and dorsal respectively.

The auricular part of the heart now expands dorsally and
laterally but more especially laterally, and comes to bulge on
either side round the conus (compare Text-fig. 9 A, B and C, A) ;
it also gradually expands on either side of the sinus venosus
ventral to the ducts of Cuvier, and overlaps the unexpanding
auricular canal anteriorly and laterally.

The posterior auricular wall, however, on which the auriculo-
ventricular ridge has appeared, takes little part in this general
enlargement, with the result that it remains comparatively
short, and the sinu-auricular and auriculo-ventricular open-
ings, which are situated at either end of it, remain com-

paratively close to one another. In Lepidosiren the auricular canal is only a transitory division of the heart externally, and early merges its identity in the auriculo-ventricular aperture; in the adult its wall is represented by a little flattened muscular band, that passes round the rim of the auriculo-ventricular opening on to the dorsal auricular wall, on which is situated the auriculo-ventricular plug.

The ventral and lateral walls of the transverse part of the heart expand rapidly (Pl. 5, figs 5 and 6, V.) while the dorsal curvature and the distal part of the ventral curvature of the heart form the proximal part of the bulbus cordis (compare Text-fig 9, A, B and C, B.). The ventricle proper is formed by the expansion of the ventral and lateral walls of the transverse part of the heart between the auriculo-ventricular and ventriculo-bulbar apertures, causing it to bulge laterally and a little posteriorly round the auricular canal and laterally and anteriorly round the proximal part of the bulbus cordis. For a time the ventricular part of the heart is considerably tilted to the left, but with the swinging of the bulbus to the front and the development of the interauricular and interventricular septa, this gradually disappears, and in the adult the ventricle is approximately symmetrically placed about the middle line (Pl. 5, figs. 5, 6 and 7, V.).

A general increase occurs in the dimensions of the bulbus cordis also, but this expansion is most marked in its middle part (Text-fig. 9A, B, and C, B.), affecting particularly the anterior and left walls of this region (Pl. 5, figs. 5 and 6, B.). The distal segment, on whose right wall the right septum has appeared reaching along the middle part as far as the proximal part of the bulbus, and on whose left wall the shorter left septum is present (Text-fig. 9C, B. R. 1 and B. R. 3.), undergoes a much lesser degree of expansion. The narrow tubular character of the proximal part of the bulbus formed by the dorsal curvature of the heart and the distal segment of the ventral curvature has already been referred to.

The bulbus as a whole, however, is now rapidly increasing

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in length, and this, combined with the localised expansion
of its middle part round the comparatively unyielding right

TEXT-FIG. 12.
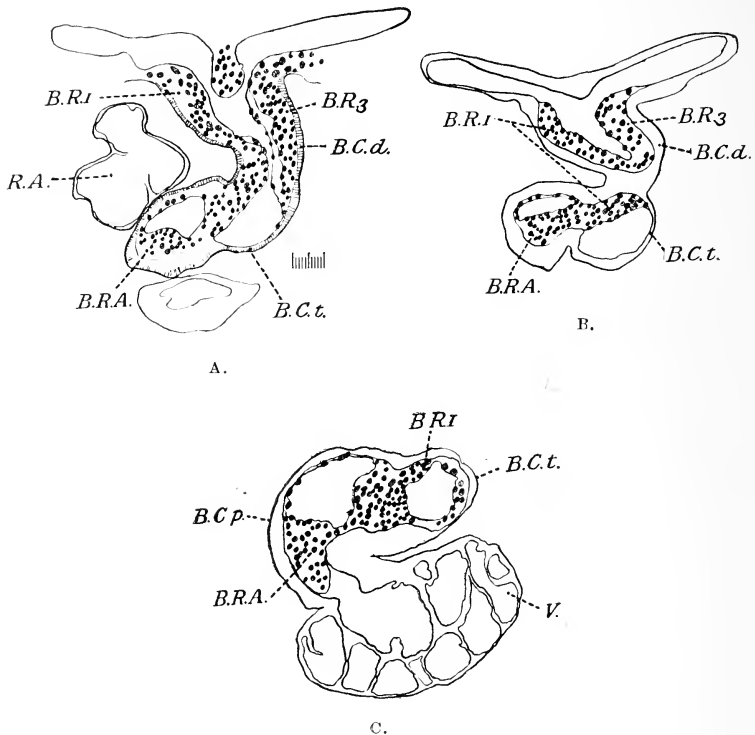
A.   Section through the bulbus cordis at Stage 32, showing
continuity of bulbus ridges 1 and A.   B. Section through
the bulbus at Stage 32, showing continuity of bulbus ridges
1 and A. along the transverse part of the bulbus.   c. Section
through the ventricle and the proximal end of the bulbus at
Stage 31, showing continuity of bulbus ridge 1 and A. along
the posterior (dorsal) wall of the transverse part of the bulbus.
$B. C. d.$ Distal segment of bulbus. $B. C. p.$ Proximal segment
of the bulbus. $B. C. t.$ Transverse segment of bulbus.
$B. R. 1.$ Right bulbus ridge. $B. R. 3.$ Left bulbus ridge.
$B. R. A.$ Ridge on anterior (ventral) wall of proximal segment
of the bulbus. $R. A.$ Right auricle. $V.$ Ventricle.

wall, causes it to kink abruptly on itself in this middle
region, and gives the expanding part the appearance of being

frilled on across the long axis of the bulbus cordis (compare Pl. 5, figs. 5, 6 and 7, *B. C. t.*, also Text-fig. 12a, b and c, *B. C. t.*). Thus a distinct bulging towards the left—the side away from the septum on the right wall—occurs, causing a marked external constriction [1] on this side between the proximal and middle parts of the bulbus (Pl. 5, figs. 5, 6 and 7, *P.*). As the bulging tapers more gradually towards its distal end, the third part of the bulbus is demarcated externally from the middle part by a less abrupt constriction[1] on the right side (Pl. 5, figs. 5, 6 and 7, *D*). This kinking and asymmetrical expansion of the middle part of the originally comparatively straight bulbus results in the formation of the transverse segment, and the original right wall of this part adopts a transverse posterior (dorsal in terms of the adult) position. Similarly, that part of the right septum situated on the right wall of this region of the bulbus becomes correspondingly displaced, and extends, transversely to the long axis of the bulbus, along what has become the posterior (dorsal) wall of the middle part (Text-fig. 12c, *B. R.* 1.). If the two ends of a straight perpendicular tube be approximated to one another so as to form a transverse kink about the middle of its length, it will be found that the right wall of the upper part is continuous with the upper wall of the transverse part, that is, with what was originally the right wall of the middle part. If now the transverse part expand forwards and upwards round its unextending upper wall, that wall—and any structure on its inner surface—will come to occupy a somewhat posterior position. This is apparently what happens in the development of the bulbus cordis of Lepidosiren. The gradual rotation of the definitive long axis of the heart into a position parallel with the long axis of the embryo must again be recalled, which, while the distal end remains fixed, swings

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[1] Proximal and distal Knickungsfurche of the developing bulbus cordis of Lacerta.—Greil, A., "Beiträge zur vergleichenden Anatomie und Entwickelungsgeschichte des Herzens und des Truncus arteriosus der Wirbeltiere," 'Morph. Jahrb.,' Bd. 31, 1903.

the proximal part of the bulbus round to the left (Pl. 5, figs. 5, 6 and 7). This, combined with the presence of the expanding auricles, probably assists in determining the bulging of the middle part mainly in an anterior direction and to the left. The bulbus has now attained its adult form, and lies cushioned against the auricles, appearing to indent them, with its posterior (dorsal) wall very intimately related to their anterior (ventral) surfaces (Pl. 5, fig. 7).

TEXT-FIG. 13.
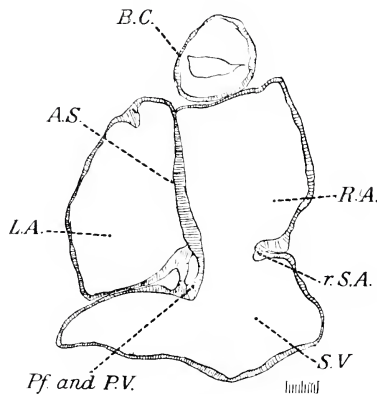
Horizontal section through the heart at Stage 32. *S. V.* Sinus venosus. *r. S. A.* Right sinu-auricular fold. *R. A.* Right auricle. *B. C.* Bulbus cordis. *A. S.* Auricular septum. *L. A.* Left auricle. *Pf.* Incipient pulmonary fold. *P. V.* Pulmonary vein.

By Stage 32 the demarcation of the various chambers from one another is achieved, and their various orifices more or less clearly defined.

The sinu-auricular opening remains, as before, a circular aperture opening into the auricular chamber on the right and having the auriculo-ventricular ridge with the developing pulmonary fold (Text-fig. 13, *P. f.*) on the left. The bulging of the right auricle along the right side of the sinus venosus, as already mentioned, gives rise to a constriction externally between the two chambers, that is represented in the interior of the heart by a fold guarding the right side of

the sinu-auricular opening (Text-fig. 13, r. S. A.). Dorsally
this fold is lost on the auricular roof, while ventrally it
curves round on to the right side of the auriculo-ventricular
ridge. The development of this right sinu-auricular fold is
therefore identical with that of the similar right venous
valve in Elasmobranchs (21).

The auriculo-ventricular aperture is defined by the marked
localised bulgings of the auricle and ventricle on either side
of the extremely short auricular canal. The auricles expand
markedly over the dorsal curvature of the heart, while the
comparatively much slighter degree of ventricular expansion
occurs wholly along the ventral curvature (Text-fig. 9 B).
This disproportion of growth results in a marked constric-
tion externally, that is manifested in the interior of the
heart by the formation of an acute auriculo-ventricular
angle or ledge, that curves round but does not quite
encircle this aperture. The ledge is most prominent where
the expanding auricle bulges over the first part of the dorsal
curvature, that is, over the dorsal wall of the proximal part
of the bulbus, thus forming the bulbo-auricular groove (Text-
fig. 9 B and Pl. 5, fig. 1, B. Ag.). On the posterior wall
this constriction and its corresponding ledge reach the sides
of the auriculo-ventricular ridge (plug), and, when the
cardiac musculature appears (Stage 30), muscular bundles—
musculature of the auricular canal—grow round this ledge
and into the ridge posteriorly from either side, thus becoming
continuous with the musculature of the developing inter-
ventricular septum. As development proceeds, the auriculo-
ventricular opening becomes somewhat horseshoe shaped
with the convexity anterior.

The bulbus also comes to be distinctly separated from the
ventricle, partly by the disproportion of their rates of expan-
sion, and partly by the increasingly abrupt curve with which
the heart is bent on itself in this region. No valvular struc-
ture, apart from the spiral fold, appears in the aperture
between the bulbus and the ventricle during any period of
development. The bulbo-ventricular opening is, from the

first, situated directly in front of the auriculo-ventricular
opening, and this relationship is permanent throughout
development.

Septa.—While these changes have been taking place in
the various chambers, defining them externally and internally
from one another, localised cellular proliferations occur that
give rise to the various septa of the adult heart. The first
of these structures to appear (Stage 27)—the auriculo-ventri-

TEXT-FIG. 14.
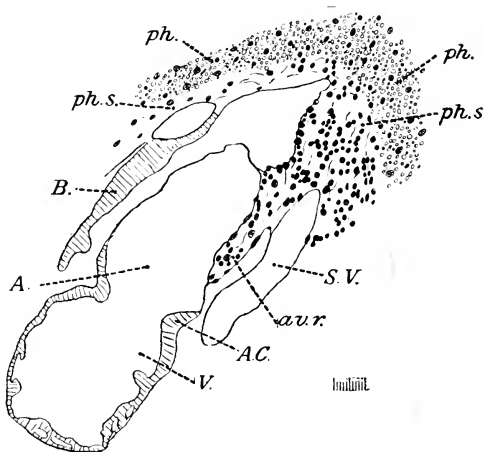
Sagittal section through the heart at Stage 30. *A.* Auricle.
A. C. Auricular canal. *av. r.* The auriculo-ventricular ridge.
B. Bulbus cordis, with left ventral aorta above. *ph.* Endo-
dermal pharyngeal rudiment. *ph. s.* Pharyngeal sheath con-
tinuous with *av. r.* *S. V.* Sinus venosus. *V.* Ventricle.

cular ridge—has already been partly described (p. 84) :
it now remains to complete that description and to consider
with it the development of the inter-auricular and inter-
ventricular septa, as well as, to a certain extent, that of the
pulmonary vein. The auriculo-ventricular ridge is intimately
related to all of these important structures, forming, as it
were, their common point of convergence.

Pulmonary Fold.—The close relationship of the two

ends of the primitive heart-tube to the pharyngeal rudiment will be recalled.

The pharyngeal rudiment (Text-fig. 14, *ph.*) extends dorsally across the narrow pericardiac cavity, and, with its sheath, remains for some time closely applied to the sinus venosus behind the pericardium (Text-fig. 14, *S. V.*). When the cellular proliferation that gives rise to the auriculo-ventricular ridge occurs, its cells are continuous with those of the pharyngeal sheath along the left sinu-auricular angle (Text-fig. 15, *av. r.* and *ph.s.*).

A little later, about Stage 31, the pharynx becomes split off from the dorsal surface of the anterior part of the sinus, but this split occurs dorsal to a little column of cells (Text-fig. 15 E, F and G, *p.v.*) lying obliquely across the roof of the sinus, and arching down anteriorly across its left wall, where it is continuous with the auriculo-ventricular ridge and the interauricular septum (Text-fig. 15 D and E, *p.v.*). The splitting-off process occurs gradually from before backwards synchronously with the development of the pharynx and lung. Posteriorly, the little column of cells remains continuous with the ventral surface of the pharyngeal and lung rudiments (Text-fig. 15 G, *ph.s.*). Finally, it becomes the scaffolding along which the common pulmonary vein crosses the roof of the sinus venosus, and arches ventrally and to the left to reach the auricle from the ventral surface of the lung (Stage 31). Once in the auricle the pulmonary vein opens through a slit in the left side of this little cell mass, dorsal to the auriculo-ventricular ridge, and the margins of the opening presently project a little, forming a hood-like fold (Text-fig. 17, *P. V.*). The pulmonary vein therefore develops in the left wall and roof of the sinus venosus, but later, owing to the expansion of the auricles and the rotation of the heart, its terminal portion comes to lie deeply between the two auricles (Text-fig. 13, *P. f.* and *P. V.*). The pulmonary fold, to which reference has been made in the account of the adult heart (Pl. 5, fig. 1, *P. f.*), is thus formed by the right wall of the terminal portion of the pulmonary vein that passes for-

TEXT-FIG. 15.
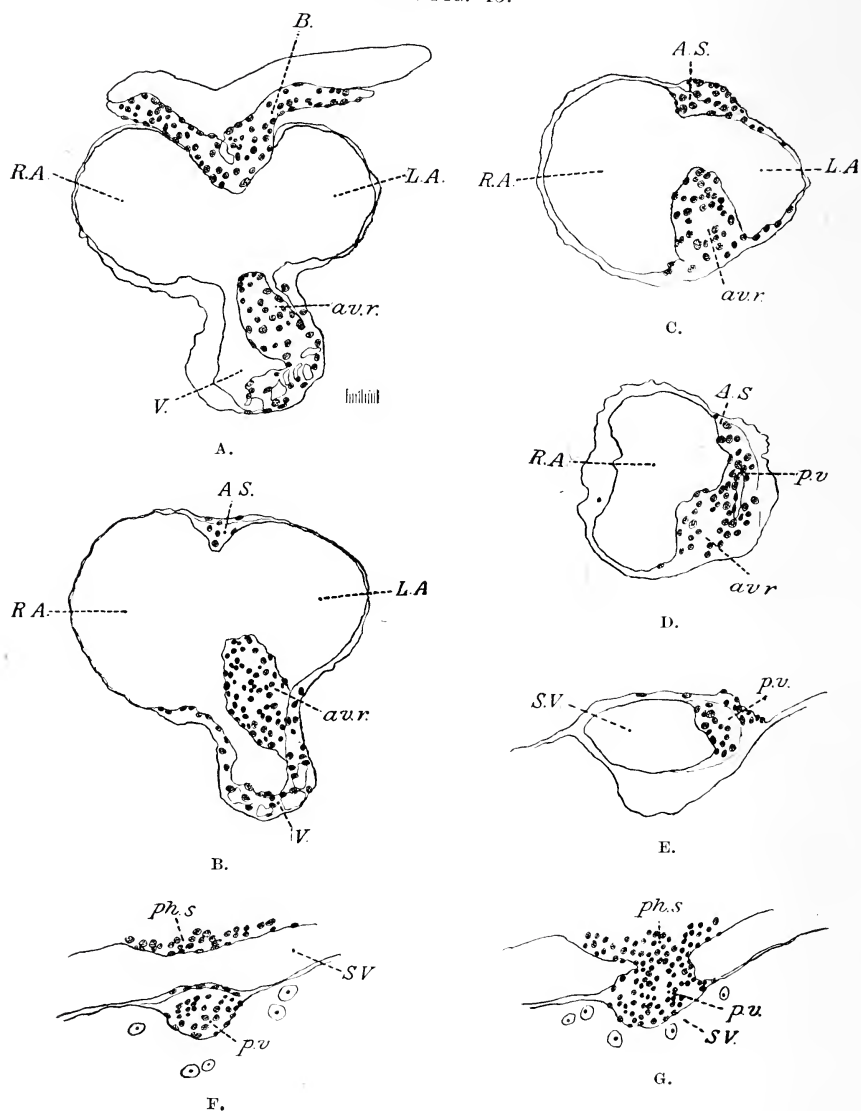
Serial sections (transverse) through the heart of Lepidosiren
at Stage 31. A. Through the anterior part of the ventricle.
Note continuity of the auriculo-ventricular ridge (*av.r.*) with
the ventricular musculature. B. Through the posterior region
of the ventricle. C. Through the auricles. D. Anterior to the

wards across the dorsal surface of the auriculo-ventricular plug into the auricular cavity. By the fusion of its anterior margin with the developing muscular trabeculæ of the inter-auricular septum (Text-fig. 15 c and d, *A. S., p, r.*) this fold comes to form part of that structure. With the development of the pulmonary vein the left auricle and ventricle expand rapidly, approximating more to the dimensions of the compartments on the right side of the heart.

Interauricular Septum. — Simultaneously with the appearance of the pulmonary fold (Stage 31), a little growth of the endothelium of the auricular roof takes place across that part of it that may be said to be pinched in the narrow space between the bulbus cordis and the sinus venosus. The little endothelial fold (Text-fig. 15B, *A. S.*), the rudiment of the interauricular septum, arches across from the posterior wall of the bulbus to the anterior termination of the rudimentary pulmonary fold and the auriculo-ventricular ridge, and there fuses with them (Text-fig. 15A, B, c and d, *A.S.*), thus in its turn also coming into relation with the auriculo-ventricular ridge. Later, fine muscular strands develop in and about this little septum, forming an irregular meshwork of fibres placed more or less parallel to the long axis of the heart, but it does not appear to attain to any marked degree of development till after the adult stages are reached.

The situation and development of the interauricular septum as well as its relations to the auriculo-ventricular ridge (plug) are identical with those of the similar septum in Urodeles

sinu-auricular junction. The inter-auricular septum (*A. S.*), the wall of the pulmonary vein (*p. v.*) and the auriculo-ventricular ridge (*av. r.*) are all three continuous in this section. E. Posterior to the sinu-auricular junction. F. Through the sinus venosus posterior to fig. E. G. Through the sinus venosus posterior to fig. F. Note continuity at this point of the column of cells (*p. v.*) on the dorsal wall of the sinus in which the pulmonary vein will develop, with the tissue of the pharyngeal and lung sheath (*ph. s.*). *A. S.* Inter-auricular septum. *av. r.* Auriculo-ventricular ridge. *B.* Bulbus. *L. A.* Left auricle. *p. v.* Column of cells situated anteriorly on the left and posteriorly on the dorsal wall of the sinus venosus, in which the pulmonary vein will develop. *ph. s.* Pharyngeal sheath. *R. A.* Right auricle. *S. V.* Sinus venosus. *V.* Ventricle.

(**12**), where the posterior (dorsal) auriculo-ventricular pocket valve is homologous with the auriculo-ventricular plug of Lepidosiren (vide infra).

Interventricular Septum.—Meanwhile the interventricular septum and musculature are also appearing (Stage 30). Little subendothelial proliferations occur (Text-figs. 11 and 16, *av. r.*), connected with the tip of the auriculo-ventricular ridge, along the posterior (dorsal) and ventral walls of the ventricle somewhat to the left side. Almost immediately, however, other little muscular buds appear on the lateral walls as well (Text-fig. 16, *m. b.*).

TEXT-FIG. 16.
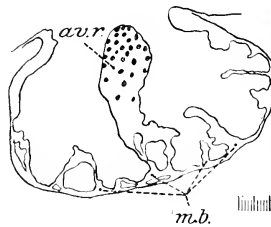
Section through the ventricle at Stage 31 showing buds of muscular tissue projecting into the ventricle. *av. r.* Auriculo-ventricular ridge. *m. b.* Muscle buds.

The last mentioned buds that are not from the first continuous with the auriculo-ventricular ridge project into the lumen of the ventricle, and as their somewhat club-shaped tips converge, sooner or later neighbouring buds coalesce, forming irregular arches and meshes which all again converge on the central "point d'appui" formed by the tip of the auriculo-ventricular ridge as it appears in the auriculo-ventricular aperture (Text-fig. 17).

These converging arches thus form trabeculæ that sweep on either side, from the posterior (dorsal) rim of the auriculo-ventricular aperture on to its lateral margins, and, mesially on to the anterior (ventral) margin of the bulbo-ventricular aperture. As the two apertures are situated antero-posteriorly,

so also are the more prominent, more mesially situated trabeculæ. Thus the interventricular septum is formed by the convergence of numerous muscular trabeculæ, from the floor and sides of the ventricle, upon the auriculo-ventricular plug. With the further growth of the heart and the elongation and expansion of the ventricles in a caudal direction, the radiating meshwork of muscular bands becomes more complicated and denser (Text-fig. 18, *V. S.*), as well as more

TEXT-FIG. 17.
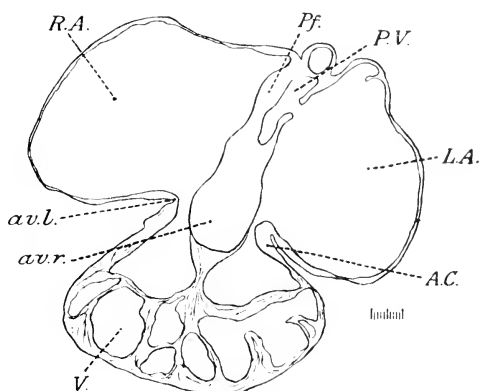
Section through the heart at Stage 32. *A. C.* Auricular canal musculature. *a. v. l.* Auriculo-ventricular ledge. *av. r.* Auriculo-ventricular ridge (plug) in auriculo-ventricular opening continuous dorsally with the pulmonary fold and ventrally with the ventricular musculature. *L. A.* Left auricle. *P. f.* Pulmonary fold. *P. V.* Opening of pulmonary vein. *R. A.* Right auricle. *V.* Ventricle.

drawn out, until finally the septum acquires the solid character of the adult condition. As this ventricular increase in size is a matter of peripheral expansion, the right and left ventricles are formed, not so much by the upgrowth of the septum as by the expansion backwards of the ventricles on either side of it. The somewhat unequal division of the ventricular cavity into a larger right and smaller left compartment—due partly to the left-sided development of the

septum (Text-fig 15 A, B, C, D, *av. r.*), partly to the compara-
tively late development of the pulmonary vein—is maintained
throughout, though in the adult the disparity is very slight.

The interventricular septum in Lepidosiren would thus
appear to be homologous with the incomplete posterior ven-
tricular septum of Lacerta, and therefore also with the
posterior muscular part of the complete interventricular
septum of the Alligator or Crocodile (**9**).

Musculature of the Heart.—From the first the dense
musculature and comparatively rigid walls of the auricular
canal and proximal part of the bulbus cordis form a marked
contrast to the rapid peripheral expansion and loose tra-
becular meshwork that characterise the ventricular and
auricular chambers of the heart. As the ventricle expands
its comparatively thin walls bulge round the compara-
tively rigid auricular canal and bulbus cordis respectively.
The result at the auriculo-ventricular aperture is that the
auricular canal has the appearance of being invaginated
into the ventricle; really the ventricle has grown up round
it. This brings the musculature of the auricular canal
into direct relation with the posterior trabeculæ of the
developing interventricular septum posteriorly, and laterally
with the endocardial surface of the ventricular musculature
round the periphery of the auriculo-ventricular opening
(Text-fig. 18, *A, C*). Anteriorly, the auricular canal comes
directly in contact with the posterior wall of the proximal
part of the bulbus at the bulbo-auricular fold (Text-fig.
9 B, *B. Ag.*) and the musculature of these two parts is
continuous round it. On the auricular side the musculature
of the auricular canal tapers off imperceptibly into that of
the auricles (Text-fig. 17, *A. C.*). At the bulbo-ventri-
cular aperture the ventricle bulges laterally and anteriorly
round the proximal part of the bulbus, so that it also projects
somewhat into the ventricle, but here the process appears to
be more strictly a folding between the two divisions of
the heart, and the ventricular and bulbar musculatures pass
into one another round the edge of the fold. The muscula-

ture of the second and third parts of the bulbus is very poorly developed.

The relations in Lepidosiren of the developing ventricular musculature to the short auricular canal and the auriculo-ventricular ridge (plug) situated in that canal, resemble closely those of the ventricular musculature to the posterior (dorsal) auriculo-ventricular pocket valve in the elasmobranch (**12**). In Lepidosiren, however, the auriculo-ventricular ridge does not become hollowed out into a typical

TEXT-FIG. 18.
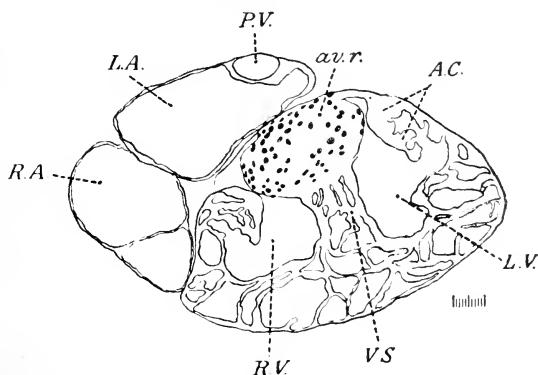
Horizontal section through the heart at Stage 32. *A. C.* Musculature of auricular canal continuous with endocardial surface of the ventricle. *av. r.* Auriculo-ventricular ridge (plug). *L. A.* Left auricle. *L. V.* Left ventricle. *P. V.* Pulmonary vein. *R. A.* Right auricle. *R. V.* Right ventricle. *V. S.* Interventricular septum.

pocket valve, and its muscular connections with the ventricular wall form the septum of that compartment of the heart.

Auriculo-ventricular Plug.—With the appearance of muscular fibres in the ventricle, the development of the interventricular septum and the definition of the auriculo-ventricular aperture, the auriculo-ventricular ridge becomes greatly thickened in its portion immediately dorsal to the auriculo-ventricular opening till it assumes the button shape that distinguishes its final form and adapts it admirably for

closing the auriculo-ventricular aperture. (Compare Text-figs. 17 and 18, *av. r.*)

Finally, the cellular matrix of which this structure is composed now develops into the cartilaginous tissue that gives it its characteristic consistency.

This auriculo-ventricular plug, therefore, originates in a little cellular proliferation at the left sinu-auricular margin. It extends along the short posterior auricular wall and lies in the auricular canal, projecting a little into the ventricular part of the heart. It is intimately related to the interventricular and interauricular septa, as well as to the pulmonary fold; throughout it is in proximity to the sinu-auricular opening and itself occupies the auriculo-ventricular aperture, while its actual area of attachment to the heart wall is the only representative in the adult Lepidosiren of the posterior auricular wall of the embryonic heart. The origin and situation of this plug would point to its homology with the posterior (dorsal) pocket valve of the auricular canal in elasmobranchs; in Lepidosiren, however, there is at no time any trace of an anterior (ventral) valve. Again, its relations to the posterior (dorsal) arch of the interauricular septum and to the opening of the pulmonary vein point to its homology also with the posterior (dorsal) auriculo-ventricular endocardial cushion of the urodele (12). Thus the auriculo-ventricular plug in Lepidosiren may be regarded as a modified auriculo-ventricular pocket valve. Boas (2) suggests that the auriculo-ventricular plug of Ceratodus, which is a structure similar in position and general relations to that of Lepidosiren, arises from the fusion of the posterior (dorsal) valves of the approximated sinu-auricular and auriculo-ventricular openings, and compares the heart of Ceratodus with that of Lepidosteus, where these two sets of valves are comparatively wide apart and quite distinct. As has been shown, the development of the auriculo-ventricular ridge in Lepidosiren distinctly supports this suggestion. The ridge appears first at the sinu-auricular junction and extends uninterruptedly along

the comparatively very short posterior auricular wall to the auriculo-ventricular junction.

Septa of the Bulbus Cordis.—At Stage 30 a thickening (Text-fig. 19, r.) is present on the ventral wall of each lateral ventral aorta, extending from a point between the origins of the common stems of the two anterior (third and fourth, Text-fig. 19, $a.\ a.\ III$ and IV) and the two posterior (fifth and sixth, Text-fig 19, $a.\ a.\ V$ and VI) afferent vessels, to the distal end of the bulbus cordis.

In the bulbus itself, these ridges ($B.\ R.\ 1.$ and $B.\ R.\ 3$) extend along the lateral walls towards its proximal end

TEXT-FIG. 19.

Section at Stage 30 transverse to the right ventral aorta at the point of divergence of the common stem of the third and fourth from that of the fifth and sixth afferent aortic vessels. $a.\ a.\ III$ and IV. Common stem of third and fourth aortic arches. $a.\ a.\ V$ and VI. Common stem of fifth and sixth aortic arches. r. Ridge of thickening on ventral wall between the two vessel stems.

(Text-fig. 9c, $B.\ R.\ 1$ and $B.\ R.\ 3$). The right ridge ($B.\ R.\ 1$) extends as far as the distal end of the proximal part of the bulbus, where it tapers off and is lost; the left ridge ($B.\ R.\ 3$) extends only about a third of the way along the left wall of the bulbus. In the proximal part of the bulbus a ridge ($B.\ R.\ A.$) also appears at the same time on the anterior (ventral in the adult) wall (Stage 31, Text-fig. 20B, c and D, $B.\ R.\ A.$).

Presently, about Stage 32, a general cellular proliferation occurs round the circumference of the whole length of the bulbus—except on the expanded middle part—which results at its distal end in the temporary appearance of two additional ridges (fig. 20A, $B.\ R.\ 2$ and $B.\ R.\ 4$) between the

first and third ridges, while in the proximal part three little cushions (fig. 20D, *en. c.*) appears on the posterior (dorsal) and lateral walls. Ridge 4 tends to become fused with ridge 1 and in two specimens could only be traced at intervals, the walls of the bulbus being occupied by the ridges 1, 2 and 3. The additional second and fourth ridges disappear with the general flattening of the bulbar lining that soon follows, but the little secondary cushions in the proximal part remain and

TEXT-FIG. 20.
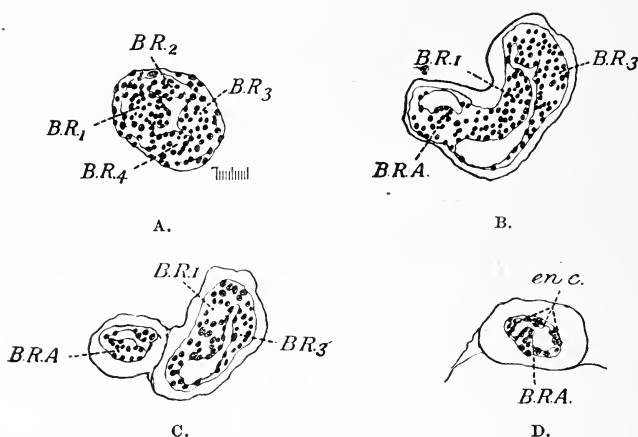
Serial sections (transverse) through the heart of Lepidosiren at Stage 34. A. Through the distal end of the bulbus cordis. B. Through the transverse segment of the bulbus. Note the continuity of the ridge (*B. R. A.*) on the anterior (ventral) wall of the proximal segment of the bulbus, along the transverse segment with the right bulbus ridge (*B. R.* 1). C. Through the posterior part of the transverse segment of the bulbus. D. Through the proximal end of the proximal segment of the bulbus. *B. R.* 1. Right bulbus ridge. *B. R.* 2. Posterior temporary ridge. *B. R.* 3. Left bulbus ridge. *B. R.* 4. anterior temporary ridge. *B. R. A.* Ridge on the anterior (ventral) wall of the proximal segment of the bulbus. *en. c.* Endocardial cushions of rudimentary pocket-valves.

are visible in the adult, where they form the rudimentary pocket-valves. The right and left ridges (*B. R.* 1 and *B. R.* 3) and the A ridge persist as the septa of the adult bulbus, ridges 1 and A forming together the spiral valve.

At the junction of the lateral ventral aortæ to form the extremely short ventral aorta, the free edges of the ridges on their ventral—posterior in the adult—walls (Text-fig. 19, r.) fuse in the middle line : the horizontal partition so formed unites at its distal extremity with the dorsal wall of the aorta, thus cutting off the fifth and sixth from the fourth and third pairs of afferent aortic vessels (Text-fig. 21, B. R. 1, B. R. 3).

Meanwhile the rotation and asymmetrical bulging of the bulbus cordis already described have taken place, with the result that the consequent kinking towards the left between its proximal and middle (transverse) segments brings the projecting free margin of the proximal extremity of the 1 ridge,

TEXT-FIG. 21.
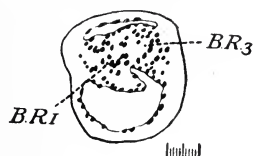
Transverse section at Stage 36 through the distal end of the bulbus cordis (ventral) aorta showing fusion of the right and left ridges or valves.   B. R. 1. Right ridge.   B. R. 3. Left ridge.

and the distal extremity of the left side of the A ridge, into close proximity round the rim of the kink between these two segments (Text-figs. 12 A, B, and C, and 20 B, B. R. 1, and B. R. A.) of the bulbus.

Consequently the two ridges fuse and form the spiral valve. This spiral valve therefore arises in Lepidosiren discontinuously in two portions, one, namely, belonging to the ventral wall of the proximal segment of the bulbus (A ridge) and one belonging originally to the right wall (B. R. 1 ridge) of the distal and middle segments. It has already been shown, however, how, in the process of development, the right wall of the middle segment was carried transversely and somewhat posteriorly and with it the valve situated on its endothelial surface. The two portions of the spiral valve,

therefore, finally come in contact with one another round the left margin of the constriction that demarcates the proximal from the middle segment. Finally, (in the adult horizontal position of the heart) the free inner borders of the first and third septa incline towards one another in the lumen of the bulbus, and the former becomes concave on its dorsal and the latter on its ventral surface (Text-fig. 22, *B. R.* 1 and *B. R.* 3.).

In the transverse part of the bulbus, the transverse continuation of septum 1 maintains this concavity, which, however, in this region is directed posteriorly (Pl. 5, figs. 1, 2, and 4, *Sp. V.t.*).

The chief interest in tracing the development of the bulbus

TEXT-FIG. 22.
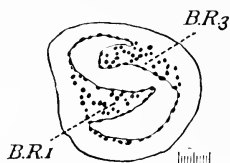
Transverse section through the distal segment of the bulbus cordis at Stage 37. *B. R.* 1. Right bulbus ridge (spiral valve). *B. R.* 3. Left bulbus ridge (left longitudinal valve.)

cordis in Lepidosiren is the information so obtained concerning the formation of the spiral valve. If the foregoing observations are correct, then—at least in Lepidosiren— the bulbus valve owes its spiral form to the process of kinking and asymmetrical expansion of an elongated but originally straight bulbus, and not to any twisting or counter-twisting of that segment of the heart. Boas (**2**) comments upon the difficulty of believing that any twisting of the bulbus (conus) occurs in Ceratodus, but having only an adult specimen at his disposal for examination cannot suggest any other more satisfactory explanation for the presence of a spiral valve. It is of interest to note how closely the above account of the development of the bulbus cordis and its associated endothelial structures agrees with that given by Langer (**16**) and Greil (**9**) for amphibians and reptiles.

When they first appear at either end of the heart, the non-muscular septa are formed by proliferations of the local mesenchyme cells, but as they grow proximally away from their points of origin into the heart itself, the endothelial cells appear to take a larger and larger part in their formation. It is extremely difficult to dogmatise, however, as to the exact derivation of these septal cells, as the mesenchyme and endothelial elements much resemble one another, but undoubted proliferation of the endothelial cells does occur at the proximal ends of the growing septa. The endothelial origin of the endocardial cushions in the elasmobranchs would point to the probability of a similar origin in Lepidosiren.

As far as any considerable changes in shape are concerned, the development of the heart may now be considered to be complete. The only change still taking place is that the ventricular and auricular walls continue for a time to become increasingly muscular.

IV. Development of the Arteries.

Aortic Arches.—The endothelial rudiments of the lateral ventral aortæ are present about Stage 24 (24 segments); they pass from the mesial heart rudiment between the parietal layer of the pericardium and the lateral expansions of the pharyngeal rudiment sharply outwards dorsally, and a little backwards to the sides of the head.

A little later, Stage 25, the rudiments of the four external gills appear on the dorso-lateral surfaces of the head, and the ventral aorta (Text-fig. 23 A, *V. A.*) on either side is prolonged outwards and dorsally to their bases, giving an afferent branch to each of the four posterior gill-rudiments, there being no afferent aortic branches to the first and second (mandibular and hyoid) arches. Correlated, however, with the flattening of the embryo and the extreme lateral position of the gill-rudiments at the sides of the neck, the origins of the four posterior aortic arches are fused together, one behind the other at the outer extremity of the paired ventral aorta
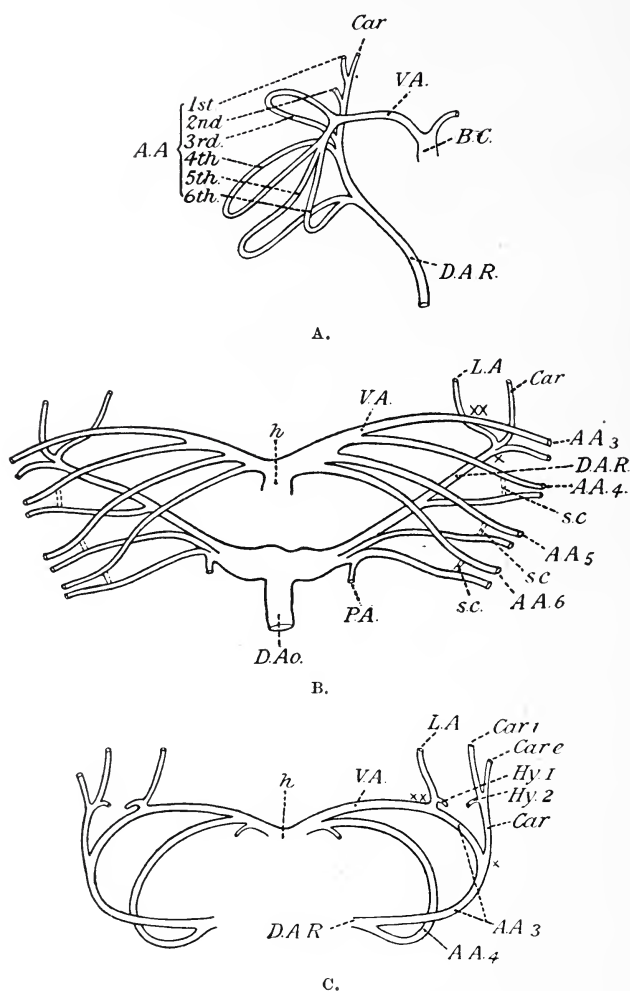
TEXT-FIG. 23.

A.

B.

C.

Diagram of the development of the aortic arches. Ventral view.
A. About Stage 26.   B. About Stage 33–34.   C. About
Stage 35.   *A. A.* The six aortic arches of which 1 and 2 are
incomplete.   *B. C.* Bulbus cordis.   *Car.* Dorsal carotid
artery, *Car. e.* External branch of carotid.   *Car. i.* In-
ternal branch of carotid.   *D. Ao.* Dorsal aorta.   *D.A.R.* Dorsal
aortic root.   *h.* Heart.   *Hy.* 1. Branch to hyoidean hemibranch.
Hy. 2. Hyoidean efferent artery.   *L. A.* Lingual artery.   *P. A.*
Pulmonary artery.   *s. c.* Short-circuiting channels between the
afferent and efferent branchial vessels.   *V. A.* Ventral aorta.

(Text-fig. 23 A, *A. A.*). These afferent branches are at first very short and each is continuous with an efferent branch that passes back along the gill, dorsal to the afferent vessel. Each efferent vessel in turn joins the dorsal aortic root (Text-fig. 23 A, *D. A. R.*), which passes from just in front of the mandibular arch, backwards and inwards towards the middle line, where ultimately it meets its fellow of the opposite side behind the heart in the region of the pronephros, forming the unpaired dorsal aorta. In the region of the mandibular and hyoid arches the dorsal aortic root receives a slender first and second efferent aortic vessel (Text-fig. 23 A, *A. A.* 1 and 2). The present observer has not been able to determine any connection between the lateral ventral aorta and these two vessels, though possibly an exceedingly fine communication may exist for a time. The ventral end of the first aortic vessel is prolonged downwards and inwards along the outer side of the mandibular arch over the ventral surface of the pericardium and terminates in the region of the cement organ. With the commencing atrophy of that organ, the little vessel breaks up and disappears about Stages 33–34. The second incomplete aortic arch, which is even more insignificant than the first, disappears still earlier.

The four posterior external gills grow out rapidly into conspicuous projecting structures on the sides of the head, and give off long feathery filaments through which the branchial vessels pass, forming a fine capillary loop in each. Subsequently with the development of the opercula, the external gills gradually disappear, and, with the establishment of the mouth-cavity and the definition of the gill-clefts, each pair of vessels becomes continuous round its respective branchial arch by means of a new short-circuiting vascular channel that develops between the dorsal and ventral (efferent and afferent) vessels (Stages 34–38, Text-fig. 23 B, *s. c.*). These new channels—where the fourth, fifth and sixth pairs of aortic arches are concerned—appear as little widening chinks between the respective vessels, but unlike what happens in urodeles (**18**), cannot be said to develop either from afferent

to efferent, or efferent to afferent sides of the arches. The short-circuiting channels of the sixth aortic arches do not expand so markedly as do those of the fifth, fourth and third. The sixth arches themselves dwindle in size, the fifth aortic arches now bringing the main supply of blood to the pulmonary vessels. The pulmonary vessels in turn, owing to the diminished calibre of the sixth aortic arches, finally have the appearance of arising directly from the dorsal aortic roots instead of from the arches themselves (Text-fig. 2, *P. A.*).

The short-circuiting of the third aortic or first branchial arches occurs somewhat differently and must be considered separately. About Stage 31 a new vascular channel appears at the junction of the third efferent aortic vessel with the dorsal aorta (Text-fig. 23 B, *L. A., x.*), and passing dorsally to and obliquely across the afferent branch of the third aortic arch in the first branchial arch, reaches the root of the tongue, and is prolonged forwards along the side of that organ (Text-fig. 23 B, *L. A.*) as the lingual artery. Later this new vessel fuses with the third aortic arch just where that vessel passes into the first branchial arch at the root of the tongue (Text-fig. 23 B, *xx.*), that is to say, just at the outer termination of the lateral ventral aorta. The third aortic arch now disappears between this point of fusion (Text-fig. 23B, *xx.*), and the point of origin of the new artery from the dorsal aorta (Text-fig. 23 B, *x.*), so that the new vessel forms the short-circuiting channel for the third aortic arch (compare figs. 23 B and 23 C, *L. A. xx.* and *x.*).

This apparent origin of the lingual artery from the dorsal aortic root must be regarded as a secondary condition due probably to the precocious development of the short-circuiting vessel of the third aortic arch. It is a temporary condition only, and with the formation of the short circuit a small vessel develops (Stage 38) at the junction of the lingual artery with the ventral aorta (Text-fig. 23 C, *Hy.* 1.), and passes outwards and backwards along a little gill-rudiment—hyoidean hemi-branch—that appears (Stage 38) in the angle between the first branchial arch and the lateral wall of the mouth. This

little gill is drained by a tiny efferent vessel which joins the dorsal carotid artery just at the point of divergence of its internal and external branches (Text-fig. 23 c, *Hy.* 2).

The presence primarily of six aortic arches, of which the two first are incomplete, having no connection with the ventral aortæ, may be compared with the early condition described for Lepidosteus (**19**). The later development of the four posterior aortic arches, however, with the formation of the lingual artery and the disappearance of the first and second arches, closely resembles the condition in Urodeles, and the lingual and dorsal carotid vessels of Lepidosiren are homologous with the external and internal carotids respectively of the former (**3**).

Dorsal and Ventral Aortæ.—The dorsal aortic roots, formed by the efferent branchial vessels on either side, join below the notochord in the region of the pronephric glomeruli to form the dorsal aorta (Stage 25, forty-seven segments) Immediately before doing so they give a branch to each glomerulus. The dorsal aorta is now present throughout the greater length of the embryo; quite anteriorly it is a patent dilated vessel, but further back it is a flattened tube, while quite posteriorly it is merely a little rod of yolky cells that are beginning to vacuolate. For a short period (Stage 26) the aorta bifurcates posteriorly and communicates with the posterior terminations of the posterior cardinal veins, while a little later (Stage 29) it extends backwards and anastomoses round the post-anal gut with the caudal vein. (A similar stage is recorded in the development of Polypterus (**15**)). These connections are soon lost, however, and finally the caudal aorta lies in the hæmal canal immediately dorsal to the caudal vein, owing to the post-anal gut having disappeared. Anteriorly with the absorption of the yolk and the general development of the embryo, the junction of the dorsal aortic roots gradually occurs further forward nearer the heart, so that finally the unpaired dorsal aorta extends some little distance in front of the pronephric region, while the length of the dorsal aortic roots is proportionately

shortened. Meanwhile, as the adult condition is attained, the long axis of the ventral aorta becomes horizontal.

Carotid Arteries.—The carotid arteries appear early as prolongations forwards of the dorsal aortic roots passing ventral to the anterior cardinal veins (Text-fig. 23 A, *Car.*). As development proceeds they come to lie below the otocysts, and give off an external branch which is distributed with the developing trigeminal nerve (Text-fig. 23 c, *Car. e.*), after which they pass sharply inwards to the ventro-lateral surfaces of the brain. An anastomosis occurs between the two internal carotid vessels below the hind brain, and then, after passing forwards a little, they anastomose on each side with the vertebro-cerebral artery (see below) round the hypophysis, and are then distributed to the mid- and fore-brains.

Vertebro-cerebral Arteries.—About Stage 28 two little vessels appear on the dorsal surface of the dorsal aortic roots passing to the ventral surface of the posterior part of the hind brain. These vessels, the vertebro-cerebral arteries, are prolonged along the ventral surface of the hind-brain and anastomose at intervals along their course (Text-fig. 2, *V.C.A*). Finally they reach well on to the dorsum of the hypophysis, forming a single median trunk, and then, separating again practically at right angles, curve round on either side and join the internal carotid vessels on the sides of the mid-brain. A little later each develops at its point of entry to the hind-brain a posterior or vertebral branch, that is continued backwards along the ventral surface of the spinal cord as far back as the origins of the first pair of segmental arteries posterior to the subclavian vessels. Posterior to this, each pair of segmental arteries supplies a twig to these vertebral vessels. Homologous vessels are described for Lacerta and Tropidonotus (1).

Cœliac Artery.—The arteries to the pronephric glomeruli appear on the formation of the dorsal aorta (Stages 24–25). They enter the glomeruli dorsally, and after winding throughout their length, leave them ventrally and disappear among

the pronephric tubules, where it is impossible to trace them among the sinuses of those structures. It is possible that the glomerular arteries may establish connections with the vitelline meshwork for a time, through the pronephric sinuses. At Stages 31–32 the developing liver comes in contact with the ventral surface of the right glomerulus and pronephric tubules and a small artery passes from the tip of the glomerulus along the right outer angle of the liver, and, crossing ventral to that organ, reaches the right wall of the developing gut. At Stage 36 with the atrophy of the glomeruli and pronephric tubules, the left glomerular artery disappears, but the right artery persists as the cœliac artery. In Lepidosiren apparently there is only one vessel to each pronephric glomerulus, and the cœliac artery receives no branches either directly or indirectly from the aorta, and only anastomoses with the anterior mesenteric artery in the walls of the intestine. There is no evidence of any shifting of the root of this vessel during development.

Pulmonary Arteries.—The pulmonary arteries appear about Stage 31 +, arising from the sixth aortic arch on either side. When the sixth aortic arch dwindles in size, as already described, the pulmonary artery comes to open from the common stem of the fifth and sixth aortic arches, receiving its main blood-supply from the fifth. With the rotation of the lungs, the right pulmonary artery comes to be distributed to the dorsal and the left to the ventral surfaces of the lungs.

The segmental aortic vessels from which the mesonephros receives its arterial blood-supply appear about Stages 31, 32, as do also the subclavian, pelvic and mesenteric arteries.

V. Development of the Veins.

Ductus Cuvieri.—At Stage 24 + (twenty-four segments) the endothelial rudiments of the vitelline veins extend, posterior to the pericardium, one on either side, across the anterior surface of the yolk, outwards and somewhat posteriorly (Text-fig. 24 A, V. V.).

TEXT-FIG. 24.
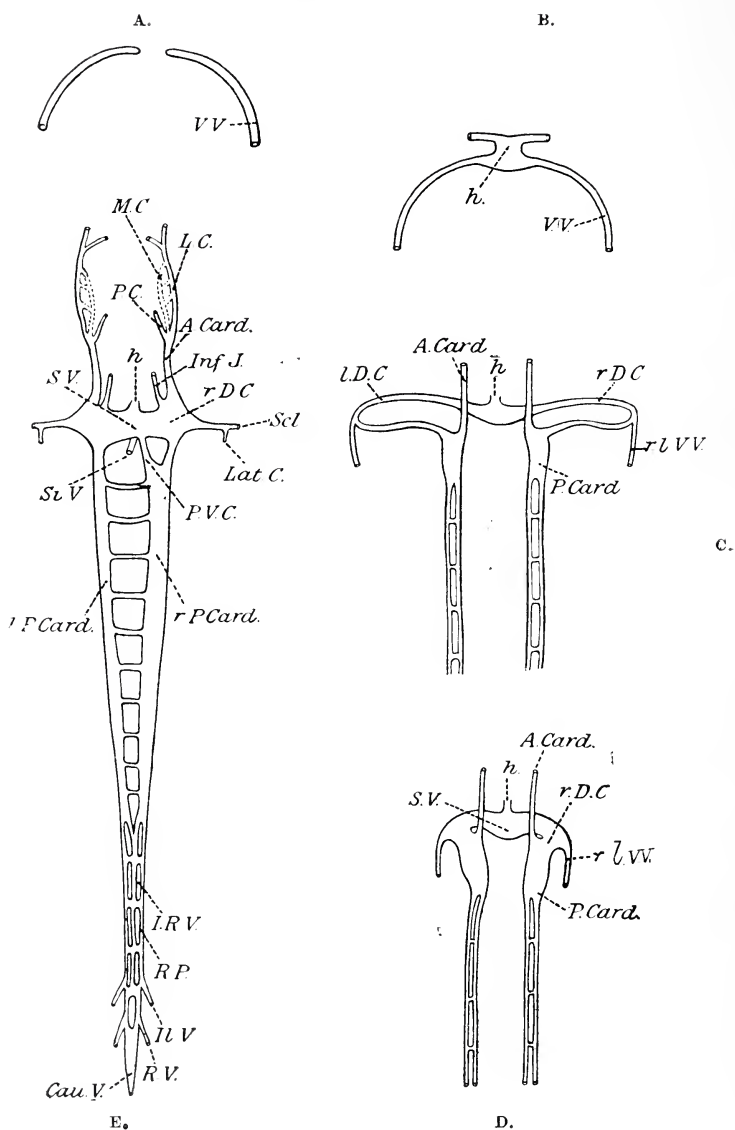
Diagrams of developing venous system, dorsal view.   A. Diagram of developing vitelline veins.   B. Diagram of junction of vitelline veins and formation of the heart.   C. and D. Diagram

A little later (Stage 24) the inner ventral ends of these endothelial vessels fuse and form the posterior part of the primitive heart-tube from which the sinus venosus is developed (Text-fig. 24 B, *V. V.*).

The peripheral ends of the vitelline veins communicate with the blood-spaces appearing on the lateral surfaces of the yolk (Stages 24–25), and simultaneously the anterior cardinal veins appear in the lateral regions of the head, passing backwards dorsal to the developing branchial vessels as far as the inner margins of the anterior ends of the developing pronephric sinuses (posterior cardinal veins) on either side. The pronephric sinuses in turn are, as in Polypterus (**15**), connected for a time at intervals along their outer margins with the vitelline meshwork; and also as in Polypterus and Belone (**22**) the most anterior of these connections curves outwards, downwards and forwards, communicating directly with the vitelline vein going to the heart and thus forming a venous arch over the lateral surface of the yolk. These venous arches, ducts of Cuvier, each receive for a time a main lateral vitelline vein from the lateral surface of the yolk, both of which vessels become modified later on with the development of the liver and sub-intestinal vein (Text-fig. 24 c, *r. D. C.* and *l. D. C.*). As the yolk is absorbed the ducts of Cuvier finally become much shortened, forming straight lateral trunks on either side of the heart, and giving rise to the sinus venosus at their point of junction behind it. With the appearance of blood-spaces in the liver (Stages 30–31) the ducts of Cuvier become much

of developing cardinal veins. E. Diagram of venous system. *A. Card.* Anterior cardinal. *Cau. V.* Caudal vein. *h.* Heart *Il. V.* Iliac vein. *Inf. J.* Inferior jugular vein. *I.R. V.* Inter-renal vein. *L. C.* Lateral cephalic section of anterior cardinal. *Lat. C.* Lateral cutaneous vein. *l. D. C.* Left ductus Cuvieri. *l. P. Card.* Left posterior cardinal. *M. C.* Obliterating median cephalic section of anterior cardinal vein. *P. Card.* Posterior cardinal vein. *P. C.* Posterior cerebral. *P. V. C.* Posterior vena cava. *R. V.* Rectal vein. *R. P.* Renal portal vein. *r. P. Card.* Right posterior cardinal vein. *r. D. C.* Right ductus Cuvieri. *r. l. V. V.* Right lateral vitelline vein. *Scl.* Subclavian. *Si. V.* Subintestinal vein. *S. V.* Sinus venosus. *V. V.* Vitelline veins.

expanded, and each forms a great venous channel extending transversely across the upper part of the anterior surface of the liver, into the outer ends of which the anterior and posterior cardinal veins open at right angles opposite one another on either side (Text-fig. 24 D, *r. D. C.*).

The posterior cardinal vein (pronephric sinuses) thus come into close relationship with the sinuses in the liver. The venous channels developing in the liver become concentrated towards the right in the neighbourhood of the sinus venosus and form a wide vessel opening into it ventrally (Stages 31–32).

Posterior Cardinal Veins and Caudal Veins.— With the development of the pronephric tubules and archinephric ducts, blood-spaces appear round them, foreshadowing the two posterior cardinal veins (Text-fig. 24 c and D, *P. Card.*). These spaces develop rapidly into considerable sinuses at the anterior ends of the tubules and communicate with the anterior cardinal veins, forming the ducts of Cuvier as described. Posteriorly these sinuses are continued along each archinephric duct as two vessel-rudiments, one on the inner dorsal and one on the outer ventral surface, which are connected round the duct by frequent anastomoses, forming the posterior cardinal veins. The posterior cardinal veins extend along the archinephric ducts to their terminations on the sides of the cloaca (Stage 24), and, passing a little ventrally and forwards, are then continuous with the blood-spaces appearing on the ventral surface of the yolk, joining the precloacal subintestinal vein, which at this stage is still extremely short. An anastomosis occurs between the posterior termination of the aorta and the posterior cardinal veins at Stage 27 (Text-fig. 25, *D. Ao.*; *P. Card.*), and the lateral trunks formed by the junction of these vessels (i. e. the posterior cardinal vein and the bifurcations of the aorta and subintestinal vein on either side) join almost immediately and form the short caudal vein ventral to the post-anal gut.

As the post-anal gut lengthens, the aorta and caudal vein

extend backwards, communicating round it by one or two vertical anastomoses (Stage 29). These post-anal vessels are remarkable for their width and form large vascular loops round the gut. With the development of the tail and the disappearance of the post-anal gut, the caudal vein lengthens and the hoop-like anastomoses with the aorta disappear, while the vein comes to lie immediately ventral to the aorta (Stage 31).[1] In the region of the cloaca, therefore, the caudal vein bifurcates to reach the posterior cardinal veins, which are joined in the same region by the bifurcations of

TEXT-FIG. 25.
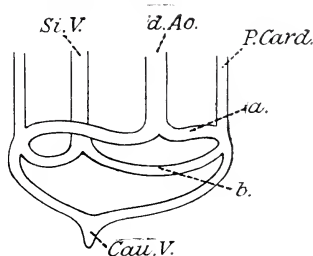
Diagram of anastomoses between the dorsal aorta and the posterior cardinal and subintestinal veins. *Cau. V.* Caudal vein. *D. Ao.* Dorsal aorta. *P. Card.* Posterior cardinal vein. *Si. V.* Subintestinal vein. *a.* The anastomosis between the dorsal aorta and the posterior cardinal vein. *b.* The anastomosis between the subintestinal and posterior cardinal veins.

the subintestinal vein. These latter vessels (the bifurcations of the subintestinal vein) presently lose their connections with the subintestinal vein and persist in the adult as the rectal veins (Text-fig. 24 E, *R. V.*). A little later (Stage 31 +) the iliac veins (Text-fig. 24 E., *Il. V.*) open into the renal portal vessels a short distance in front of the cloaca.

Meanwhile, with the gradual absorption of the yolk, the archinephric ducts approach one another in the middle line, and, with the appearance of the mesonephros dorsal and

[1] A similar condition is described in the development of Polyterus. Kerr, J. Graham, "The Development of Polypterus senegalus, Cuv." The work of John Samuel Budgett, Cambridge, 1907.

immediately internal to them, the outer and inner channels of the posterior part of each posterior cardinal vein are separated to some extent. Later the inner trunks meet and fuse temporarily, forming a median interrenal vessel (Text-fig. 24 E, *I. R. V.*), while the outer trunks form the renal portal vessels (Text-fig. 24 E, *R. P.*). In front of the mesonephros the outer vascular channels disappear, becoming apparently fused with the inner channels. Numerous anastomoses occur between the inner and outer trunks through the substance of the mesonephros.

The development of the posterior cardinal veins up to this point thus closely resembles that of the same vessels in the Urodeles (**11**), with this difference, that in Lepidosiren the fusion of the two posterior cardinal veins across the middle line is only a temporary, not a permanent condition.

At the same time (Stage 31) changes occur in the posterior cardinal veins cranial to the mesonephros. The right posterior cardinal becomes the larger of the two vessels, and transverse anastomoses appear segmentally between them as far forwards as the region of the pronephric glomeruli (Text-fig. 24 E, *r.* and *l. P. Card.*). The main blood-stream is thus deflected to the right and a large new vascular channel appears in the mesentery of the liver. This new channel arises on the right at the level of the most anterior anastomosis between the posterior cardinal veins (Text-fig. 24 E, *P. V. C.*), and passes forwards in the triangle formed by the pronephros on the right, the lung rudiment on the left and the yolk and liver ventrally. Where it passes from the surface of the yolk on to that of the liver, the anterior termination of this vessel is continued into the wide channel formed by the liver sinuses and subintestinal vein and so enters the sinus venosus (Stage 31), while posteriorly it is in continuity with the wide trunk of the right posterior cardinal vein, thus forming the posterior vena cava. The front part of the posterior vena cava appears in the mesentery of the liver, on the ventral surface of the lung rudiment ventral to the common pulmonary vein. This close relationship is maintained throughout

development, the latter vessel resting on the dorsal surface of the former in the adult. The development of the posterior vena cava, as just described, is very similar to the condition in Urodeles (11), where the new vessel connects the fused portions of the posterior cardinal veins (which, however, separate again in Lepidosiren) directly with the heart through the liver.

As the lung and foregut extend backwards, they form a barrier between the left posterior cardinal vein and the posterior vena cava (right posterior cardinal), so that their transverse anastomoses gradually disappear from before backwards, even the fused portions of the posterior cardinal veins in the posterior mesonephric region (interrenal vein) are wedged apart again, and in the adult only one or two transverse anastomoses persist across the middle line towards the posterior end of the body (compare Text-figs. 24 E and 3). At the same time the two posterior cardinal vessels lose their connection with the renal portal vessels at the caudal extremity of the mesonephros, and no longer receive blood from the caudal vein. Finally, therefore, the main inner channels on the inner dorsal surfaces of the mesonephros are formed by the left posterior cardinal and the posterior vena cava (right posterior cardinal) respectively. With the atrophy of the pronephros the anterior part of the right posterior cardinal vein loses its connection with the posterior vena cava in the region of the pronephric glomerulus (compare Text-figs. 24 E and 3, *r. P. Card.*), and is represented in the adult by a short vessel that receives one or two small tributaries from the body-wall and a vein from the vertebral region, and joins the anterior cardinal vein to form the right duct of Cuvier. The left posterior vein persists as a long, somewhat slender trunk passing forwards from the left mesonephros between the intestine and the body-wall to the left duct of Cuvier (Text-figs. 24 E and 3, *l. P. Card.*).

Anterior Cardinal Veins.—The anterior cardinal veins appear early in the lateral regions of the head (Stage 25), arching backwards dorsal to the branchial vessels, and

communicating with the ducts of Cuvier at their junction with the pronephric sinuses (posterior cardinal veins, Text-figs. 24 c and d, *A. Card.*). The anterior ends of these vessels bifurcate in the region of the eye, one branch being superficial, and one, the anterior cerebral vein, passing deeply from the front part of the head. As they pass backwards the anterior cardinal veins lie ventral to the otocysts and then curve inwards internally to the posterior cranial nerves, and then outwards again a little to reach the pronephric sinuses. Presently, however, a new vessel appears on either side below and slightly external to the otocysts. This new vessel arises from the anterior cardinal vein immediately anterior to the ganglion of the seventh and eighth cranial nerves, and, passing immediately external to it, joins the anterior cardinal vein again between the eighth and ninth nerves (Stage 30) A little later (Stage 31) the outer vessel grows further back externally to the ninth nerve, and then joins the anterior cardinal vein between the ninth and tenth nerves, while finally (Stage 31+) it extends backwards external to the tenth nerve and joins the anterior cardinal vein behind it. Meanwhile, as each fresh segment of this lateral cephalic vessel develops, the corresponding stretch of anterior cardinal vein (median cephalic) disappears from before backwards (Text-fig. 24 e, *M. C.*). The posterior part of the third segment of the median cephalic persists, however, in the adult as a short wide vessel between the skull and muscles of the head, opening with the posterior cerebral vein from the interior of the skull, into the lateral cephalic vein behind the otocyst. Finally, the lateral cephalic vein passes below the otocyst and external to the cranial nerves, being continuous with the anterior cardinal vein in front of and behind them and forming to all appearances simply a portion of the anterior cardinal. In Lepidosiren therefore, while the main features of the development of the anterior cardinal veins are the same as for all vertebrates, the details tally closely with those given for the same vessels in Tropidonotus (**10**).

Posterior Cerebral Veins.—At Stage 28 a small vessel from the brain opens into each anterior cardinal vein some distance posterior to the otocysts. A little later (Stage 31 +) the posterior anastomosis of the lateral cephalic vein with the anterior cardinal occurs just in this region, and, when the median cephalic part of the latter vessel atrophies, the cerebral vein (Text-fig. 24 E, *P. C.*) and the short persistent posterior part of the median cephalic open together into the lateral cephalic vein. This relationship persists in the adult, the cerebral vessel forming the posterior cerebral branch of the anterior cardinal vein.

Lateral Vitelline and Subintestinal Veins.—When the primitive vitelline veins become connected over the lateral surfaces of the yolk with the anterior and posterior cardinals so that they may now be termed the ducts of Cuvier (Text-fig. 24 C, *D. C.*), they also become connected with the general vitelline meshwork by means of two main lateral vessels one on either side (Text-fig. 24 C, *r. l. V. V.*). These lateral vitelline veins extend backwards and ventrally over the sides of the yolk towards the anus, their tributary vessels forming intricate anastomoses all over its surface : in the region of the anus they fuse and form the, at first extremely short, subintestinal vein (Text-fig. 26 A, *lat. V. V., Si. V.*).

A little later (Stage 32) three main vessels can be traced over the yolk towards the heart, viz. two lateral and one smaller median vessel (Text-fig. 26 B, *r.* and *l. lat. V. V., Si. V.*).

The appearance of this median vessel by short-circuiting through the network on the yolk, synchronises with the development of blood-sinuses in the liver (Stage 31), which form a wide vessel, opening ventrally into the sinus venosus on the right side. The median vitelline or subintestinal vein is continuous with the liver-sinuses over the antero-ventral surface of the yolk, the former channel thus obtaining access to the heart. With the further development of the embryo, and the definition of the liver and gut, the right lateral vitelline vein disappears, and the left vessel
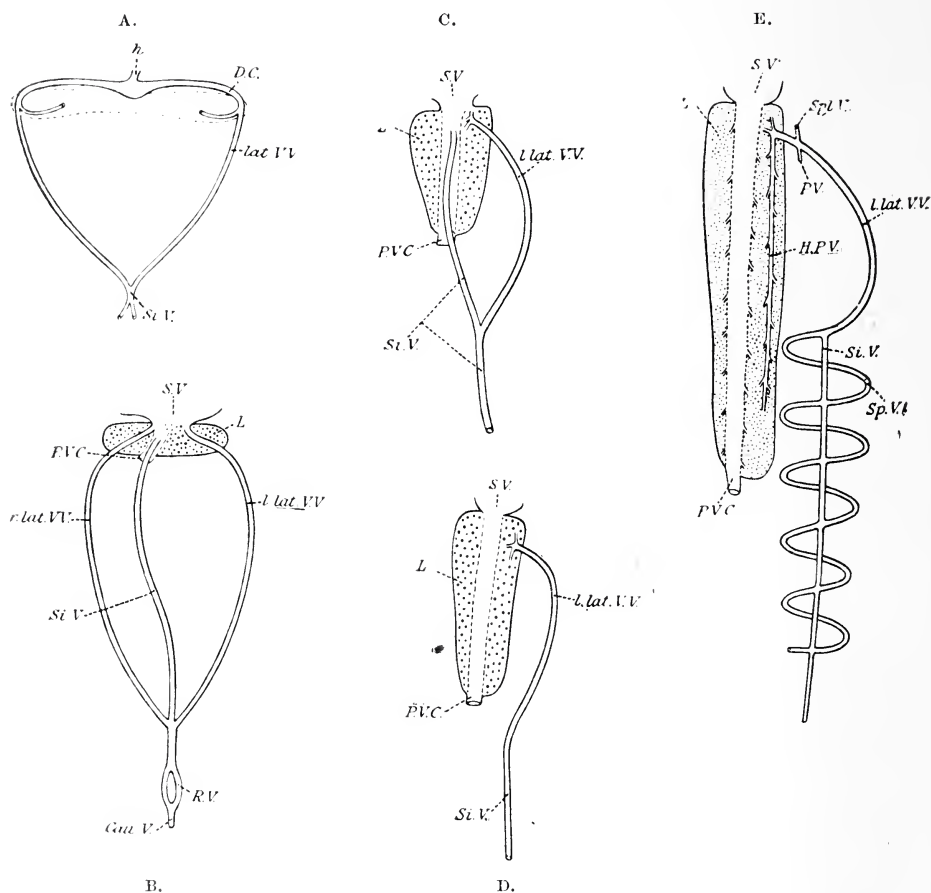
TEXT-FIG. 26.

Diagram illustrating the development of the lateral vitelline and subintestinal veins and of the portal system. A. Diagram of lateral vitelline veins. The approximate site of appearance of the liver is indicated by the dotted line. B, C and D. Diagrams of lateral vitelline and subintestinal veins. E. Diagram of the portal system. *Cau. V.* Caudal vein. *D. C.* Duct of Cuvier. *h.* Heart. *H. P. V.* Hepatic portal vessel. *L.* Liver. *lat. V. V.* Lateral vitelline vein. *P. V.* Pancreatic vein. *P. V. C.* Posterior vena cava. *r.* and *l. lat. V. V.* Right and left lateral vitelline vein. *R. V.* Rectal vein. *Si. V.* subintestinal vein. *Spl. V.* Splenic vein. *Sp. V.* Spiral vein. *S. V.* Sinus venosus.

loses its direct communication with the sinus venosus and breaks up into capillaries in the liver substance (Text-fig. 26 c, *l. lat. V. V.*).

At the same time the left lateral vitelline vein becomes connected with the venous spaces appearing in the spleen and pancreas, while it is still continuous round the dorsal wall and left side of the gut with the subintestinal vein. The anterior or hepatic segment of the subintestinal vein now disappears (Stage 35) as far back as its junction with with the left vitelline vein, so that the hinder persistent part of the subintestinal vessel (Text-fig. 26 D, *Si. V.*) loses its direct connection with the liver and sinus venosus, which it now reaches by means of its anastomosis round the left side of the gut with the left vitelline vein (Text-fig. 26 D, *l. lat. V. V.*). The lateral vitelline vein arches round the gut parallel with the insertion of the spiral valve, and at its junction ventrally with the subintestinal vein a tributary vein (Text-fig. 26 E, *Sp. V.*) emerges on the opposite side of that vessel, and passing round the gut, follows the line of insertion of the spiral valve and fuses with the subintestinal vein at each point where it crosses the mid-ventral line.

This vein of the spiral valve therefore forms a series of diminishing spiral coils that are united ventrally by the subintestinal vein (Text-fig. 26 E, *Si. V., Sp. V.*). Behind the spiral valve the subintestinal vein tapers off in the region of the anus. The portal system is thus formed by the left lateral vitelline and subintestinal veins and their tributaries that pass into the left side of the liver anteriorly, where they form a vascular channel extending along the left margin of that organ (Text-fig. 26 E, *H. P. V.*). This lateral channel appears with the formation of the portal system, and, passing back along the margin of the liver, breaks up into capillaries in its substance, from which numerous hepatic radicles convey the blood to the posterior vena cava. The development of the subintestinal (portal) vein with its tributary vessel from the spiral fold essentially resembles that of the same two vessels in the elasmobranch (**20**), except that

in the elasmobranch it is the left half of the venous ring circling the gut, not the right, that disappears.

Sinus Venosus.—The sinus venosus is formed by the junction of the ducts of Cuvier, and comes early to lie on the right of the auricle, opening on the right side of the auriculo-ventricular plug—that is to say, it expands more at the expense of the right than of the left duct of Cuvier, so that the latter remains relatively the longer vessel. With the final development of the liver and the posterior vena cava and the disappearance of the lateral vitelline and subintestinal veins, the sinus venosus becomes drawn out posteriorly and so acquires the irregular pear-shaped formation of the adult chamber. The sinu-auricular aperture and the development of the pulmonary vein and fold and their relations to the sinus have already been described in detail; it will suffice to re-state that the pulmonary vein passes from right to left in the roof of the sinus, and arches down obliquely to the left in a fold across its anterior wall to reach the left auricle. In the adult the sinus venosus is dorsal to the heart, and its ventral, anterior and lateral walls have a covering of pericardium while its dorsal wall is continuous with that structure. The closing of the pericardiac space in front of the liver behind the heart causes a contraction externally dividing the posterior vena cava from the sinus venosus. This contraction is represented in the interior of the sinus by a little projecting ledge. The subintestinal and lateral vitelline veins open into the sinus only for a short period, and the ducts of Cuvier, the posterior vena cava and the coronary vein are the only vessels permanently connected with it.

Inferior Jugular, Subclavian and Lateral Cutaneous Veins.—The inferior jugular veins appear at Stage 31 as little vessels passing ventrally to the branchial arches, backwards along the lateral surfaces of the pericardium, and opening on to the ventral surfaces of the anterior cardinal veins at the outer ends of the ducts of Cuvier (Text-fig. 24 E, *Inf. J.*). These vessels are homologous with those of the same name in the Urodeles. The subclavian veins appear

a little later (Stage 31 +), passing inwards from the rudi-
mentary pectoral limbs and entering the anterior part of the
pronephric sinuses on their outer sides (Text-fig. 24 E, *Scl.*).
As the pronephric tubules and the outer part of their sinuses
atrophy, the subclavian veins come to open into the outer
sides of the proximal part of the anterior cardinal veins
approximately opposite the entrance to the anterior jugular
vessels. In the adult, owing partly to the straightening of
the proximal parts of the anterior cardinal veins, which are
at first directed laterally, and also partly to the marked
elongation of the body in Lepidosiren, the inferior jugular
and subclavian veins appear to enter comparatively far
forwards along the anterior cardinal vessels (Text-fig. 3, *Inf.
J.* and *Scl.*). At Stage 32 two small lateral cutaneous veins
appear one on either side, opening into the subclavian veins
from behind (Text-fig. 24 E, *Lat. C.*). They pass a little
distance backwards in the superficial layers of the body-wall
and receive two or three small veins through the body
musculature from the vertebral region.

Coronary Vein.—The coronary vein appears on the
right outer wall of the ventricle while that surface of the
heart is still applied against the sinus venosus posteriorly
(Stages 31–32)—that is to say, while the heart loop is still
transverse to the axis of the embryo. The little vessel opens
from the surface of the ventricle into the sinus, and as the
long axis of the ventricle becomes parallel with that of the
body, the vein is considerably stretched and carried on to
the posterior surface of the ventricle. With the heart in the
adult position the coronary vein is on its right dorsal surface,
and opens on to the floor of the sinus venosus behind a little
fold whose free margin is directed posteriorly.

VI. Origin of Vessel- and Blood-Cells.

Vessel-cells.—At Stage 23 the lateral mesoderm is
delaminating over the surface of the yolk. The rather
columnar cells of the splanchnic mesoderm in the cardiac

region can already be distinguished; the cœlomic chinks are just appearing, and, lying between the columnar layer and the endoderm are the comparatively large, flattened, heavily yolked cells, similar to the young mesenchyme cells that later form the rudiments of the vitelline veins.

These vessel-cells, therefore, appear synchronously with the definition from the yolk or endoderm of that part of the splanchnic mesoderm that will, later, constitute the myocar-

TEXT-FIG. 27.
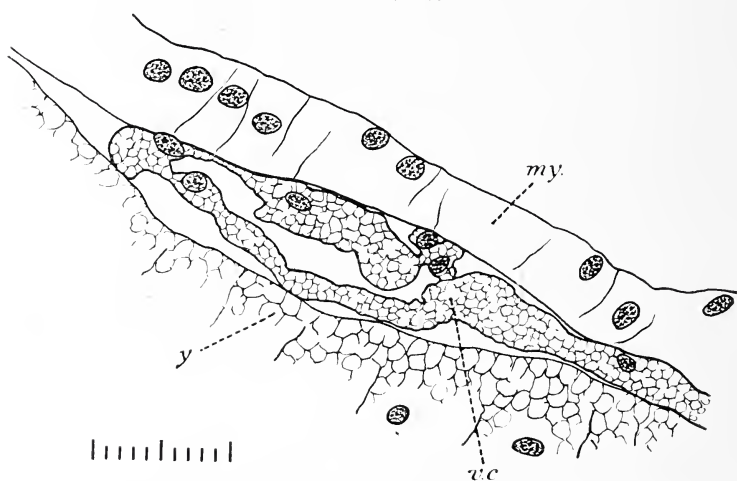
Section showing vacuolation and fusion of adjacent vessel-cells.
my. Myocardiac layer. *v. c.* Vessel-cells. *y.* Yolk.

dium: they are closely wedged between the mesoderm and endoderm, and, posterior to the cardiac area, they merge into the ordinary cells over the yolk. A little later the endothelial vitelline vessels are formed from the vessel-cells between the yolk and the myocardiac mesoderm, apparently by a process of intra-cellular vacuolation combined with a syncytial fusion of adjacent cells and the budding off into the lumen of the vessel of free blood-cells from the interlacing branches of the syncytium (Text-fig. 27, *v. c.*).

The endothelial cells of the lateral ventral aortæ are similarly formed, while, once the ventral aortæ extend

TEXT-FIG. 28.
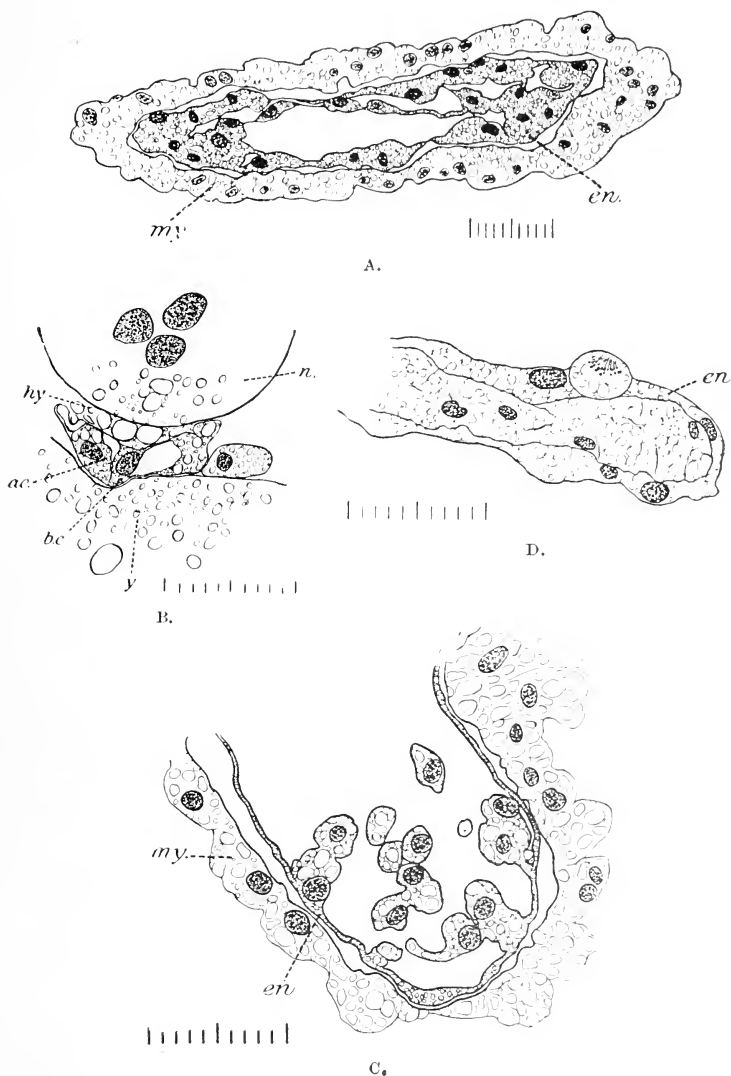
A. Transverse section through the heart showing syncytial condition of the endocardium and free cells being separated from it. B. Section transverse to aorta at Stage 23 showing vacuolation and fusion of the endothelial cells with corpuscle about to be shed into the lumen of the vessel. C. Sagittal section through heart at Stage 26, showing corpuscle being shed from the endothelium. D. Right sinu-auricular angle. Endothelial cell being shed into lumen of heart. *ao.* Aortic endothelial cells. *b. c.* Corpuscle. *en.* Endocardial syncytium. *en.* Endothelium. *hy.* Hypocordal cells. *my.* Myocardium. *n.* Notochord. *y.* Yolk.

beyond the margins of the endodermic pharyngeal rudiment, their walls are formed directly by the canalisation of the mesenchyme cells of the head region. The posterior cardinal veins and the glomerular arteries are formed from the mesenchyme layer already mentioned as lying between the

TEXT-FIG. 29.
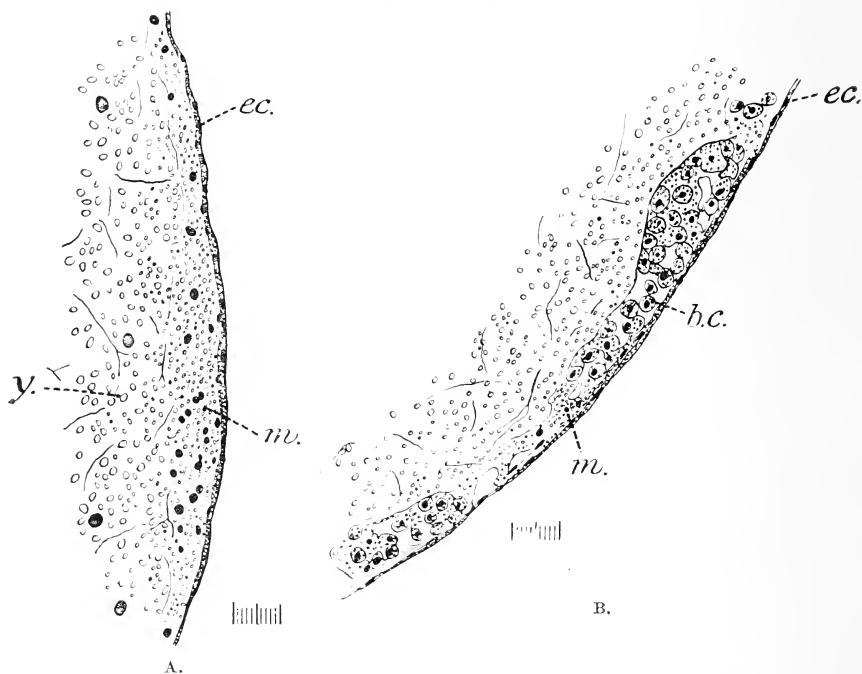
A. Section at the periphery of the yolk showing the mesoderm layers (characterised by smaller nuclei and finely fragmented yolk-granules), ill-defined from the yolk, which has large scattered nuclei and large granules. B. Section at periphery of the yolk showing the formation of the free blood-corpuscles in the mesoderm layers. *b. c.* Primitive blood-corpuscles. *ec.* Ectoderm. *m.* Mesoderm.

pronephros and the yolk, while the dorsal aorta is formed by sclerotome cells that pass in from either side ventral to the hypocorda (Text-fig. 28 B, *ao.*) In all these situations probably the young endothelial cells continue for a time to

Text-fig. 30.

A. High-power drawing of section similar to Text-fig. 29 A.
B and C. High-power drawings of sections similar to Text-fig.
29 B.   *bc.* Primitive blood-corpuscles.   *ec.* Ectoderm.   *m*
Mesoderm.   *y.* Yolk.

shed free rounded blood-cells into the lumen of the developing vessels. In the smaller vessels the actual shedding is difficult to detect, but the process is evident enough in the heart (Text-fig. 28 A, C and D, *en.*)

Blood-cells.—On the surface of the yolk the lateral mesoderm is at first only to be distinguished from the underlying endoderm or yolk-cells by the increased number of somewhat smaller nuclei and the more finely fragmented

TEXT-FIG. 31.
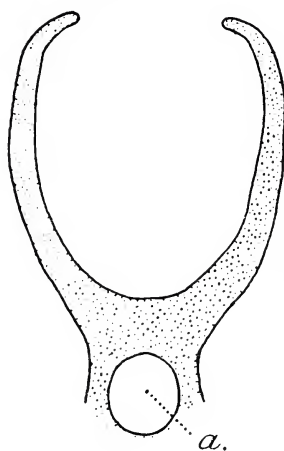
Diagram of a plane surface projection of the cardio-anal bands of multinuclear mesoderm. *a.* anus.

condition of the yolk-granules (Text-fig. 29 A, *m.*) The nuclei are generally arranged in one, but here and there in two or even three irregular layers, while the cell margins are not sharply defined, the appearance being that of multinuclear masses of yolk-laden protoplasm (Text-fig. 30 A, *m.*)

The doubling or trebling of the nuclear layer occurs mostly in little scattered patches over the surface of the yolk, but there is one continuous conspicuous band of two or even three nuclear layers (Text-figs. 29A and 30A, *m.*) in thickness on each side along a line extending from the cardiac region backwards and ventrally to the anus, where it merges

in the thicker ventral mesoderm layer. Projected on a plane surface, this multinuclear region of the lateral mesoderm would have somewhat the outline of Text-fig. 31.

The mesoderm layer over the yolk now gives rise by repeated nuclear division to a thin outer layer of splanchnic mesothelium, and internal to this, to free rounded cells—the primitive blood-corpuscles—contained in irregular lacunar spaces formed by the outer walls of the syncytial masses (Text-fig. 30B, bc.)

The formation of blood-cells is most active where the mesoderm layer is thickest, and is therefore most noticeable in the scattered areas over the yolk and along the cardio-anal lines already mentioned. In these regions cell-nests of varying sizes are formed, packed with round blood-cells floating in fluid plasma (Text-fig. 30 B and C, bc.)

These cell-nests communicate, forming irregular channels, and finally, the vitelline meshwork is established, bringing the whole extent of the vascular mesoderm elements into relation with one another. Anteriorly the vitelline system communicates with the veins along the margins of the yolk and caudally with the aorta and caudal vein.

These observations on the development of the elements of the blood-vascular system in Lepidosiren point entirely to their being of mesodermic origin (**6**).

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DESCRIPTION OF PLATE 5.

Illustrating Dr. J. I. Robertson's Memoir on the "Development of the Heart and Vascular System of Lepidosiren Paradoxa."

REFERENCE LETTERS.

A A. Third, fourth, fifth and sixth, the four persistent aortic arches. *A. S.* Interauricular septum. *A. V. pl.* Auriculo-ventricular plug in auriculo-ventricular opening. *a. b. c.* Three rows of vestigial pocket valves, each row having three valves with traces of smaller irregular valves between them. *B. Ag.* Bulbo-auricular groove. *B. C. d.* Distal segment of bulbus cordis. *B. C. p.* Proximal segment of bulbus cordis. *B. C. t.* Transverse segment of bulbus cordis. *B. R. 3.* Left longitudinal valve in the distal segment of the bulbus cordis. *b.* Dorsal row of vestigial valves in the proximal segment of the bulbus. *C. V.* Cut ends of coronary vein. *D.* Constriction between *B.C.t.* and *B.C.d.* *L. A.* Left auricle. *l.D.C.* Left ductus cuvieri. *P. B.* pericardiac band. *P.* Constriction between *B. C. t.* and *B. C. p.* *Per.* Pericardium. *P. f.* Pulmonary fold guarding opening of pulmonary vein. *P.V.* Pulmonary vein. *P. V. C.* Entrance of the posterior vena cava to the sinus venosus. *R. A.* Right auricle. *r. D. C.* Right ductus Cuvieri. *r. S. A.* Right sinu-auricular fold; the arrow indicates the sinu-auricular aperture. *S. Ao.* Prominence of the ventral aortic septum, which is formed by the fusion of the left longitudinal and spiral valves in the extremely short ventral aorta. *Sp. V. d.* Distal part of spiral valve. *Sp. V. p.* Proximal part of spiral valve. *Sp. V. t.* Transverse part of the spiral valve. *S. V.* Sinus venosus. *t. f.* Transverse furrows at the base of the spiral valve showing traces of valvular pocketings. *V.* Ventricle. *V. B. o.* Bulbo-ventricular orifice indicated by arrowhead. *V. S.* Interventricular septum.

Fig. 1.—Drawing of the adult heart from the right side magnified about 3½ times. The right walls of the right auricle and ventricle and of the proximal part of the bulbus cordis have been removed to show

the internal structures.   The sinu-auricular aperture is indicated by
an arrow that is seen against the pulmonary fold.

Fig. 2.—Drawing of the heart from the left side, magnified about $3\frac{1}{2}$
times.   The left walls of the left auricle, ventricle and bulbus cordis
have been removed.   The opening of the pulmonary vein ($P.V.$) into
the left auricle is indicated by an arrow, as is also that of the bulbus
from the ventricle ($V. B. o.$).

Fig. 3.—Drawing of the proximal segment of the bulbus cordis,
magnified about 5 times.   The dorsal wall has been cut open a little to
the right of the middle line, so that the dorsal row of vestigial valves
has been divided unequally.   No part of the bulbus wall has been
removed.

Fig. 4.—Drawing of the bulbus cordis from the ventricular side
magnified about $3\frac{1}{2}$ times.   The ventral wall of the bulbus has been
removed in the distal and transverse segment.

Fig. 5.—Drawing of the heart (magnified about 50 times) about
Stage 31.   The transverse position of the heart is still distinct.

Fig. 6.—Drawing of heart (magnified about 50 times) about Stage
33.   Owing to the various growth adjustments the transverse position
of the heart is rapidly being lost.

Fig. 7.—Drawing of heart (magnified about 50 times) about Stage 35.
The position of the heart is now practically that of the adult condition.

The Reproductive Cycle in the Marsupial Dasyurus viverrinus.

By

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With Plates 6 to 8.

CONTENTS.

INTRODUCTION.

IN recent years a great deal of attention has been paid to the reproductive processes in the Mammalia, and in particular to the phenomena connected with the œstral cycle. Observations relating to the latter, however, have been almost entirely based on Eutherian mammals, and, indeed, largely on Primates (Heape (**8, 9, 10**), Van Herwerden (**12**), Hitschmann and Adler (**15**), and others) and various domesticated or semi-domesticated Eutheria (e.g. sheep (**16**), dog (**19**), ferret (**17**), Marshall). Having at our disposal a large volume of records relating to the breeding habits of Dasyurus, as well as an abundant supply of material, we have thought that an inquiry into the reproductive cycle in this member of the Marsupialia (in many respects a more primitive group than the Eutheria), might not only be of interest, but might, perchance, throw light on some of the problems relating to the Eutherian œstrous cycle which still await solution. We venture to hope that the account of the reproductive cycle which we are now able to present will be found to fulfil, in some measure, expectations in this latter regard.

We desire to say here that our work has been greatly facilitated by the circumstance that some of the phenomena relating to reproduction in Dasyurus have already been described in greater or less detail (Hill (**13, 14**), O'Donoghue (**20, 21**), Sandes (**22**)).

The breeding season has been divided up into periods for the

purposes of description, and the terminology employed is, with slight modification, that suggested by Heape (**11**). As the œstral cycle in Dasyurus differs considerably from that of the Eutherian mammal, it has been found necessary to introduce two new terms, viz. P o s t - œ s t r u s, to designate the period which intervenes between œstrus and ovulation ; and P s e u d o - p r e g n a n c y, to designate the period which, in the non-pregnant animal, follows ovulation, and in which the changes in the ovary, mammary glands and uteri are essentially similar to those in the pregnant female.

We wish to express our thanks to Mr. F. Pittock, of the Zoological Department of this College, for invaluable help in the preparation of the photomicrographs on Plates 6–8.

Material.

The information relating to the breeding habits of the animal was obtained largely from the records mentioned above. These records relate to 170 females, which fall into two classes—pregnant and non-pregnant. Of the non-pregnant females killed, 13 were prior to ovulation, 19 were after ovulation, and in 6 there was no record of ovulation. Of the pregnant animals, 37 had less than twenty embryos, 35 had more than twenty embryos, in 25 there was no definite record of the number of embryos, and 35 were post-partum. Examples of individual records relating to these females have been given previously (**20**), and further examples are given later.

For the purposes of the present paper, the uteri of sixteen females were cut in serial section in order to study the histological changes occurring therein. These uteri were, with two exceptions, from non-pregnant animals both before and after ovulation, and were fixed either in picro-corrosive-acetic acid, strong Flemming's fluid, or in Hermann's fluid, all of which gave very good fixation, Flemming's fluid being particularly good for the cilia in the uterine glands. The sections (about $8\,\mu$ thick) were stained with hæmatoxylin

and eosin. These sections were compared with those of pregnant and post-partum uteri already in the possession of one of us, whilst we also had access to the numerous preparations of ovaries and mammary glands of Dasyurus, which formed the basis of the papers of O'Donoghue (**20, 21**) and Sandes (**22**) on the corpus luteum and the growth of the mammary apparatus. We are thus in the fortunate position of being able to take into consideration and to correlate the changes which occur during the œstral and pregnancy cycles in the several parts of the reproductive and accessory organs more accurately and in greater detail than, we believe, has yet been done for any Eutherian mammal.

Anœstrus.

The Australian native cat, Dasyurus viverrinus, is a small marsupial somewhat resembling a civet in external appearance. It is readily obtainable and fairly easy to keep alive and breed from in captivity.

In the female, the pouch[1] is a well-marked structure which, during the anœstral period, appears as a small, somewhat circular depression, situated in the median line towards the posterior end of the abdomen. It is about 10 mm. in diameter by 5 mm. in depth and its boundary is slightly less marked at the anterior end. The interior of the pouch, which in the resting animal is dry and dirty, contains as a rule six teats (vide **20**). The teats are arranged in pairs on either side of the middle line and each teat has a thickened ledge around its base. The skin over the teat itself is generally free from the sebaceous glands characteristic of the skin as a whole, and which are particularly well marked in the lining of the pouch. Examination of sections through the teat shows that the ledge around its base marks the place where the sebaceous glands of the pouch leave off. The teat with its ledge is situated in a depression, which, in its turn, is surrounded by a raised ridge very nearly circular in shape.

[1] c.f. O. Katz, 'Zitschr. wiss.,' Bd. xxxvi, 1882.

The ridges around the posterior pair of teats are continuous in the middle line, whereas those at the anterior end are separated from one another by a considerable interval. There is also a space between the median walls of the ridges of the middle pair of teats, so that the three pairs are approximately arranged in the form of a horse-shoe with the open end situated anteriorly (see Text-fig. 1, A). The floor of the pouch between the ridges surrounding the teats is loose and folded and frequently falls into a raised fold along the middle line.

The number of teats is subject to slight variation; thus in

TEXT-FIG. 1.

A   B
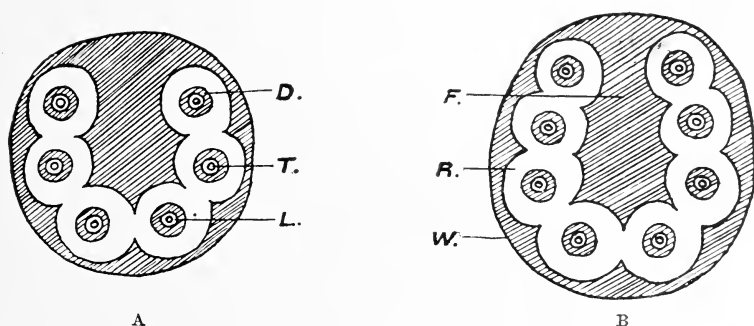
Diagram to show the arrangement of the teats within the pouch. (A) in the normal female with six teats; (B) in the females with eight teats. T. Teat. L. Ledge surrounding teat. D. Depression. R. Raised ridge. F. Floor of pouch. W. Pouch-wall.

over 170 females examined one pouch contained five teats, two pouches contained seven teats and five contained eight teats[1], but all the remainder had the normal number—six.

According to Bresslau (4, p. 672 et seq.) the mammary (nipple) primordia take their origin in Dasyurus as in other Marsupials from a pair of laterally situated primary anlagen, each consisting of an epidermal thickening and an underlying area of condensed cutis. As a rule, three pairs of mammary primordia are formed, three from each primary anlage, but

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[1] Klatsch (quoted by Bresslau (4)) also records the occurrence of 8 teats in one individual.

in two pouch-young Bresslau records the presence of four pairs, whilst in a third he found three primordia on one side, four on the other. In the pouch-young, the mammary primordia are arranged symmetrically in two slightly curved longitudinal rows, the two primordia of the posterior pair lying nearest the middle line, those of the anterior pair most remote from it, so that the two rows already show the same horseshoe shaped arrangement as is characteristic of the adult.

Round each mammary primordium, which has meantime grown into the cutis as a knob-shaped epidermal projection, a ring-shaped epidermal thickening arises and this later hollows out to form a circular groove—the marsupial pocket— bounded by a circular wall. The lateral portions of the walls of the three pockets on each side then join so as to form a continuous lateral pouch fold and finally the two folds become continuous with each other in front and behind, and so produce the circular wall of the pouch. The occurrence of supernumerary nipples (Hypermasty, Hyperthely) in D. viverrinus is of interest in view of the fact that D. hallucatus, according to Oldfield Thomas (23) normally possesses eight teats, that species of Phascologale and Sminthopsis (which genera are regarded by various authorities as being more primitive than Dasyurus), have ten, whilst higher numbers still are met with amongst the Didelphzidæ (Peramyshenseli, 17–25). These facts, as Bresslau (4, p. 805–8) has pointed out, would appear to indicate that Dasyurus, like many other marsupials, has suffered, in the course of phylogeny, a reduction in the number of teats within the pouch, which reduction has affected those more anteriorly situated. In the case of the pouch with only five teats, it seems as if six mammary rudiments were included in the pouch, but for some reason or other the anterior one on the right side failed to develop.

When the young are born, they are transferred to the pouch, presumably by the mother with the aid of her lips, and, as they become permanently fixed to the teats for some time, the number of teats determines the maximum number of young that can be reared in one litter.

Dasyurus has only one breeding season[1] in the year, which extends over the winter months, i. e. from the end of May to the first fortnight in August.

In our records there are two cases of females ovulating in May; one killed on the 21st had eggs which had just entered the uteri, and the other, killed on the 31st, showed corpora lutea in a very early stage. The two latest records of pregnant females are on the 2nd and 6th of August, and in both cases the embryos were in an advanced condition.

The male does not appear to experience an obvious period of rut, such as Semon (**25**) describes for the male Phascolarctos.   We have, however, frequently observed that the substitution of another male for the one previously with a female frequently resulted in copulation (cf. record, p. 151). The records show that copulation may extend intermittently over a period of two to three days.   In the majority of cases, however, there is only a single record of coition, generally lasting for several hours.

The method of copulation in Dasyurus is similar to that described by Selenka for Didelphys (**23**, p. 105), the male mounting on the back of the female and laying hold of the skin of the dorsum of the neck with his jaws.   The penis, when erected, is extremely long and attenuated and possesses a markedly bifid glans, its two divisions doubtless being inserted into the corresponding lateral vaginal canals.   The records show that the spermatozoa may remain alive in the Fallopian tubes (where they occur in large bunches in the gland lumina opening into same) for at least two weeks.

PRO-ŒSTRUS.

So far as our observations extend, there is no evidence of any animal ovulating twice in the same season, nor is there

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[1] This term is used in the sense defined by Heape (**11**), i.e. "to denote the whole of that consecutive period during which any male or female mammal is concerned in the production of young," and does not include the period of suckling.

any record of a female once served, again copulating during
that season.   As a matter of fact, our experience has been
that a female, once copulation has been effected, will invari-
ably fight any male subsequently introduced into the breeding
cage.   Moreover, study of the ovaries in section has shown
that ovulation, even in females which have not become preg-
nant, is not followed by the growth of a second batch of
follicles.   The œstral cycle in Dasyurus is thus a simple one,
and as only one such cycle occurs in the breeding season, the
animal is monœstrous.   Our records show that Trichosurus,
Phascolarctos (cf. Semon (**25**) and Caldwell) (**5**)), Phasco-
lomys and probably the Macropods (cf. Caldwell, loc. cit.)
breed only once a year, but as to whether they are monœs-
trous, we have no definite evidence of our own to offer.

Pro-œstrus commences early in June and appears to
occupy a period of time varying from four or five to perhaps
as long as ten or twelve days.   It is marked by an œdematous
swelling of the lips of the cloacal aperture, and at the same
time the pouch enlarges somewhat and becomes tumid, whilst
its interior sometimes becomes slightly moist.   The tumidity
is mainly the result of the enlargement of the sebaceous glands
of the pouch area.   The sweat-glands also hypertrophy and
become more coiled.   The sebaceous and the sweat-glands soon
become active; in the former the cells start to undergo auto-
lytic disintegration, and in the latter they become granular.
The gland lumina increase and become filled by secretion,
which, on being discharged, causes the interior of the pouch
to become moist and somewhat sticky (vide **20**).

Practically no change takes place in the mammary glands
during this period.

In the ovary, the Graafian follicles gradually enlarge, and
as they approach maturity, they form prominences on its
surface.

Uterine Changes.

We have examined sections of the uteri of three females in
the pro-œstral condition.

Case 1 (17. vi. '99) (Pl. 6, fig. 1).—No record beyond a label in the bottle stating that this female was "probably getting into heat." The above date shows that she was killed towards the beginning of the breeding season. The uteri measured 11 mm. by 7·5 mm. by 7 mm. in diameter. Sections show that, apart from slight increase in size and in vascularity, they are practically in the resting condition. The mucosa averages 1·4 mm. in thickness. The uterine epithelium consists of low columnar cells with close-set nuclei, and has a thickness of ·012 mm.

The uterine glands vary in diameter from ·032–·048 mm. They are distinctly coiled in the basal half of the mucosa and possess small but distinct lumina. The connective tissue matrix of the mucosa is for the most part dense and compact. Numbers of leucocytes occur in it. The blood-vessels extend up between the glands and are already considerably enlarged.

Case 2 (No. 2, 21. v. '03) (Pl. 6, fig. 2).—This female was killed on arrival. The pouch appeared reddish and was slightly moist. In the ovary occurred practically full-grown ova, with peripheral vesicular nuclei. The mucosa averages about 1·5 mm. in thickness and the uterine epithelium, ·016 mm. The uterine glands are in a more active condition than in Case 1. They are more markedly coiled, are thicker and possess wider lumina, but the mucosa as a whole is less vascular.

Case 3 (22. vi. '01).—No record beyond statement "getting into heat." Left uterus, 11 mm. by 11 mm. by 6 mm. Ova with peripheral vesicular nuclei and not quite full-grown. Graafian follicles small, with retinacula.

The mucosa has a maximum thickness of 2·2 mm. The uterine epithelium has increased somewhat in thickness, now measuring ·02 mm. and is evidently in active growth, mitoses being not infrequently met with. The nuclei of its cells are narrow, elongated and closely packed, and situated at different levels.

The basal two-thirds of the uterine glands are markedly

coiled and form compact elongate masses, separated from each other by narrow strands of connective tissue carrying blood-vessels. The lumina of the glands are only occasionally patent. The gland epithelium is in active growth, mitoses being frequent.

The connective tissue, especially in the upper third of the mucosa, is loose and œdematous in character and has numerous leucocytes dispersed through it. Immediately below the uterine epithelium is a narrow dense zone. The blood-vessels are enlarging, the mucosa being more vascular than in Case 2.

From the foregoing, it is clear that during pro-œstrus the uteri as a whole enlarge, the mucosa increases in thickness and becomes more vascular, whilst the uterine glands grow in length and become markedly coiled. The uterine epithelium also thickens.

ŒSTRUS.

Pro-œstrus is followed by œstrus, i. e. the period of desire on the part of the female, and although in one case this extended over three days, it appears usually to last only for one or perhaps two days. As in the higher mammals, it is only at this time that coition occurs. Selenka (**23**, p. 104), speaking of Didelphys marsupialis, states that, " die Brunst des Weibchens dauert jedesmal nur 3–5 Stunden ! "

Our records show that whilst in one case [1] ovulation may have coincided with œstrus, the latter practically invariably precedes the former. We have records of five females in which copulation had occurred (in three of them, five days previously), whilst ovulation had not yet taken place, as shown by examination of the ovaries. Furthermore, we have records showing that unsegmented ova were obtained " from the uteri, four (2-celled eggs also present along with unsegmented), five, six, seven and eight days after coitus, 2-celled eggs six and seven days after, 4-celled eggs eleven

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[1] This case is referred to in detail on p. 149.

and eighteen days after " (Hill, **14**, p. 3).   It is thus evident
that ovulation is not generally coincident with œstrus, but
follows after a longer or shorter interval, amounting in some
cases to six or seven or even more days.

Dasyurus thus affords a marked contrast to the Eutheria,
in the majority of which œstrus and ovulation are stated to
be generally coincident (cf. Marshall (**18**), p. 135 et seq.).

During this period the changes in the ovary and pouch
still continue.

Uterine Changes.

Case 1 (12. vi. '02), Pl. 7, fig. 3.—Female killed on
arrival.   Pouch, reddish, not moist, and only slightly tumid.
Cloacal margins distinctly swollen and tumid.   Lateral
vaginal canals, uteri and Fallopian tubes enlarged, the uteri
measuring 15 mm. × 13 mm.   No trace of semen.   Ovaries
with prominent Graafian follicles, ova full-grown with
peripheral nuclei, shortly before first polar mitosis.

The mucosa has a maximum thickness of 2·5 mm.   The
uterine epithelium (·04 mm. in thickness) is formed of very
narrow columnar cells with, for the most part, basally situated
nuclei, alternating in arrangement.   The uterine glands
(·048–·06 mm. in diameter) are markedly convoluted over the
major portion of their extent.   Their epithelial walls are
formed of columnar cells with basally situated nuclei and
with their inner ends distinctly ciliated, the cilia working
outwards (Pl. 8, fig. 10).   Cilia would appear to be present
over the entire extent of the gland lumina, though in some
cases they are less distinct and perhaps absent at the basal
extremities of the glands.   We have observed this ciliated
condition of the uterine glands also in Perameles.   It is a
feature of interest inasmuch as it affords an instance of a
rather uncommon condition, viz. a glandular epithelium, com-
posed of similar cells uniformly ciliated.

The connective tissue, except immediately below the uterine
epithelium, where it is dense and very cellular, is in the form

of a very delicate reticulum, the meshes of which are occupied by a lightly staining coagulum. Leucocytes are present throughout the extent of the mucosa, being especially abundant in its more superficial region, below the uterine epithelium.

The mucosa is not specially vascular.

Case 2 (9.vi.'01).—Female killed on arrival. Pouch with reddish hairs. Not cleaned and not much enlarged. Left uterus 12·5 mm. by 12·5 mm. by 7 mm., right uterus 14 mm. by 16·5 mm. by 7 mm. Ovaries with prominent Graafian follicles, ova full grown with peripheral nuclei. It is noted that this female would not associate with the male. Copulation may therefore have already taken place, but the genital organs were not examined for sperms.

The uterus agrees closely as regards its histological condition with that of the preceding case. The mucosa measures in thickness from 2·4 to 2·7 mm., and the uterine epithelium ·032 mm. The glands generally resemble those of Case 1 and are ciliated.

An important advance is seen in the presence of numerous capillaries in the connective tissue close below the uterine epithelium, some of which lie in actual contact with the deep surface of the latter.

Making allowance for individual variation these two cases show that the growth changes initiated during pro-œstrus are continued without interruption throughout this period.

Post-œstrus.

In the higher mammals, œstrus marks the climax of the pro-œstral changes, and is, according to Heape (11) and Marshall (loc. cit.), the period at which ovulation occurs. It is followed in the non-pregnant female by metœstrum, a period during which the various parts of the reproductive and accessory organs return to a condition of rest.

In Dasyurus, however, we meet with a markedly different state of affairs, since we find, as pointed out above, that ovulation as a general rule does not take place until some days

after œstrus. For this period which intervenes between œstrus and ovulation, we propose the term "post-œstrus."

During this post-œstral period the changes initiated during pro-œstrus are continued, and are perhaps most marked in the ovary. The Graafian follicles continue to enlarge. The ova undergo the first meiotic division, resulting in the separation of the first polar body, and the spindle for the second division is laid down. Our records yield instances of two females, both killed five days after copulation, in one of which the first polar spindle was established, whilst in the other, the first polar body was already separated and the second polar spindle formed. These ovarian changes culminate in the rupture of the follicle and the discharge of the ovum.

The tumidity of the cloacal lips gradually disappears.

Uterine Changes.

Case 1 (No. 8, 23 . vii . '02) (Pl. 6, fig. 4).—This female came to hand on July 11th. On the 18th the cloacal margins were greatly swollen, and copulation, extending over two hours, took place. Five days after, i. e. on the 23rd, she was killed, the pouch being small and only slightly tumid. The uteri were pale in colour and somewhat enlarged, measuring 16·5 mm. by 12 mm. by 5·5 mm. Examination of the ovary revealed the presence of ripe follicles containing ova in which the first polar spindle was already formed.

The mucosa has a maximum thickness of 2·4 mm. and the uterine epithelium, ·032–·036 mm. The uterine glands have increased somewhat in diameter, and some of them are markedly enlarged, especially towards their basal ends. Where such enlargement has occurred the gland epithelium has become reduced in thickness and appears of the low columnar type. In a few of the glands the lumen is occupied by a shrunken coagulum. The gland epithelium is ciliated.

The connective tissue differs from that of œstral Case 1 in being much more cellular and compact. Leucocytes are specially abundant in the superficial portion of the mucosa,

Capillaries are present here and there below the uterine epithelium, but are not specially abundant.

C a s e 2 (No. 14, 26 . vii . '02).—The following is our notebook record of this female :

24 . vi . '02.—Received.

4 . vii . '02.—Cloacal margins commencing to swell.

11 . vii . '02.—Cloacal margins still swollen.

21 . vii . '02.—Copulation.

24 . vii . '02.—Pouch slightly tumid, not moist.

26 . vii . '02.—Pouch slightly tumid; killed, i. e. five days after copulation.

Examination of the ovaries revealed the presence of mature follicles containing ripe ova in which the first polar body was already separated and the second polar spindle established.

The mucosa has a maximum thickness of 2·4 mm. The uterine epithelium has made little or no progress, and appears as a low columnar epithelium (·02 mm. in thickness) with close-set ovalish nuclei. Here and there, between the ordinary epithelial cells, there occur single very narrow cells with darkly staining cytoplasm and compressed deeply staining nuclei.

The uterine glands (averaging ·048 mm. in diameter) do not differ essentially from those of œstral Case 2, and are rather less advanced than those of the preceding uterus. They are well convoluted and lined by the usual ciliated columnar epithelium. Their lumina are for the most part distinct but small.

The connective tissue is more œdematous than that of the preceding case, and contains in places much coagulum. Numerous leucocytes are present in it. The mucosa is, on the whole, more vascular than that of Case 1, though the superficial capillaries are not yet greatly developed.

So far as can be judged from these two cases, it would appear that during this post-œstral period, no very marked advance is made by the uterine mucosa, the uteri being, on the whole, very similar to those of the œstral period.

Ovulation.

It has been pointed out by Ancel and Bouin (1) that the Eutherian mammals may be divided into two classes according to the conditions under which they ovulate. In the first group ovulation is spontaneous—i. e. it takes place whether there has been coition or not (this condition appears to be the more general); whilst in the second group ovulation is non-spontaneous—i. e. in the normal course of events, copulation is necessary to provoke it.

In Dasyurus, ovulation is spontaneous and quite independent of copulation. We have records of nine females which ovulated after being under observation for periods varying from two to thirty-seven days, during which no copulation was seen to take place. Four of these females were under observation for twenty days and upwards, whilst in three of them, the unfertilised ova were found in the uteri. On the other hand, as pointed out above, we have records of five animals in which copulation had taken place but ovulation had not, three of these females having been killed five days after copulation. This evidence shows conclusively that ovulation and copulation are perfectly independent one of the other, and that the former is in no way provoked by the latter, as is the case, for example, in the rabbit.

A further point in connection with ovulation is worthy of attention, although it has already been noted elsewhere (Hill (14)), and that is the remarkable number of eggs discharged from the ovary at each ovulation. In one case twenty-eight eggs, in two others thirty eggs, and in yet another thirty-five vesicles were obtained from the two uteri. "There can be no doubt that Dasyurus, like various other marsupials—e. g. Perameles, Macropus, etc.—has suffered a progressive reduction in the number of young reared, but, even making due allowance for that, the excess in production over requirements would still be remarkable enough "[1] (Hill (14)).

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[1] Examination of the ovaries of D. maculatus with full-grown follicles shows that the same excessive production of ova holds true also for this species.

Ovulation may be succeeded by one of two states. On the one hand, if the ova be fertilised, there follows a period of pregnancy, which in its turn is succeeded by a nursing period. On the other hand, in the absence of copulation or fertilisation, there follows an interval in which the various organs undergo a series of marked alterations, essentially of the same nature as those normally undergone in the pregnant and post-partum female. To this period, therefore, we propose to give the name of "pseudo-pregnancy."

Pregnancy.

If fertilisation is effected, ovulation is normally succeeded by pregnancy. The ova are fertilised in the upper parts of the Fallopian tubes, and the second polar body is there given off. They pass down the tubes, apparently with considerable rapidity, into the uteri, where cleavage begins (Hill (**14**)).

We have already directed attention to the fact " that there is no correlation between the number of ova shed during ovulation and the accommodation available in the pouch (Hill (**14**)), the number of ova shed at one period being, as a rule, far in excess of the normal number of teats (six). Our records show that out of seventy-two pregnant females in which there is a definite record of the number of eggs or embryos, thirty-five had more than twenty, and the remaining thirty-seven less than twenty. Moreover, whilst amongst the thirty-five one had as many as thirty-five vesicles, in only three of the remaining thirty-seven were there less than six embryos, and in one of these only one uterus was pregnant. Although a proportion of the eggs may for one reason or other fail to develop normally, it would appear to happen generally that a larger number of young are born than can possibly survive, the pouch accommodation being strictly limited. Our records afford two specific instances of females which were examined shortly after parturition. In one of these, eighteen young were found, of which eleven occurred adhering to the hairs round and directly below the opening of the pouch, six were attached one to each nipple, and one

occurred free in the pouch alongside an attached one. In the other, ten young were found, six attached, three free in the pouch, and one outside considerably shrivelled.

Gestation Period.

Attention has already been called to the fact that ovulation is entirely independent of copulation, a fact which renders it extremely difficult, if not impossible, to determine accurately the length of the gestation period. One of us has already recorded that "the shortest period observed between coitus and the birth of the young was a little over eight days," hence it was concluded "that the time of gestation does not exceed this period" (**14**). Selenka, it may be noted, gives the gestation period of Didelphys as barely eight days (**23**). As regards our eight-day record, we think it is desirable to give further details. Copulation was observed on June 7th, about ten a.m. It lasted a comparatively short time. The female was killed on June 15th at six p.m. A single young one (recently born, and measuring 7·5 mm. greatest length in the fresh state) was found in the pouch, which was greatly enlarged and possessed prominent sebaceous glands. Our notebook record continues, "assuming that copulation had not taken place before receipt of this female, we can put down the gestation period as a little over eight days." There are two points in this record which should be noted : first, the brevity of the copulation, and second, the question of copulation previous to receipt. The recollection of one of us regarding this particular female is that she was brought in by the collector, placed at once with the male, a short copulation, or an attempt at the same, ensuing almost immediately.

In view of the impossibility of entirely excluding the occurrence of copulation previously, we cannot regard the foregoing evidence as absolutely conclusive as to the length of the gestation period. At the same time it should not be forgotten that a female once served will not under normal circumstances again copulate. It is of course quite possible

that the circumstances in this particular case were n o t normal, since this female was no doubt in a cowed and frightened condition when placed in the cage with the male.

We have another record of a female[1] in which the young were born sixteen days after copulation. This particular female was received on June 20th. Copulation occurred on the 24th, and parturition on July 10th. This record we regard as perfectly trustworthy. The question as to when ovulation occurred, however, cannot be answered with any degree of accuracy. We have already stated that ovulation occurs at a variable period after œstrus. In our records we find that the shortest time intervening between copulation and the finding of unsegmented eggs in the uteri is four days (in this particular case the eggs were in the unsegmented and 2-celled stages), and the longest time, eight days. Between these limits we have records of the following: five days after, no ovulation (three cases); five days after, unsegmented ova (one case); five days after, unsegmented and 2-celled eggs (one case); five days after, 4-celled eggs (one case); six days after, unsegmented ova (one case); six days after, 2-celled eggs (one case); seven days after, unsegmented ova (one case).

The average interval between copulation and ovulation would thus appear to vary round about five or six days. If, now, we subtract five from the sixteen-day record given above, we have a gestation period of about eleven days. From the evidence we think we are justified in stating that the gestation period in Dasyurus is not less than eight, and does not exceed fourteen days.

General Changes.

The ovarian changes following ovulation, i. e. those result-ing in the formation of the corpora lutea, have been fully

[1] This female is the one previously referred to (ante, p. 148) as having given birth to eighteen young.

dealt with by Sandes (**22**). It need only here be mentioned that the corpora lutea essentially resemble those of Eutheria in their mode of development and structure. Sandes (p. 380) states that "the corpus luteum forms quickly, within three days [after ovulation], and persists [not only throughout pregnancy but] during the greater part of the time that the animal is lactating, ultimately disappearing when the young animal is capable of leading an independent existence." The changes in the pouch, including its sebaceous, sweat, and mammary glands have been described elsewhere (O'Donoghue (**20**)), whilst a preliminary account of the arrangement of the fœtal membranes, the placentation, and the mode of parturition, has also been published (Hill (**13**)).

Early Pregnant Uteri.

Case 1 (No. 15, 19 . vii . '01) (Pl. 6, fig. 5).—We give here for purposes of comparison a brief account of the uterus of a pregnant female with ova at the stage before the separation of the yolk-body and shortly after entering the uteri.

The record of this female is a very complete one, extending over a period of forty-one days. We reproduce it here:

8 . vi . '01.—Resting; so on to 6 . vii . '01.

9 . vii . '01.—Cloacal margins very slightly swollen.

10 . vii . '01.—Fresh male in; no copulation.

11 . vii . '02.—Cloacal margins swollen, but not greatly.

13 . vii . '02.—Cloacal margins swollen. Fresh male in; copulation.

17 . vii . '02.—Pouch hardly altered; cloacal swelling still present.

18 . vii . '02.—Pouch very slightly tumid, very dirty.

19 . vii . '02.—Pouch distinctly tumid, not cleaned.

Killed, i. e. six days after copulation.

The uteri measured 1·3 cm. by 1·3 cm. by ·7 cm., the left containing ten ova and the right fifteen.

The mucosa has a maximum thickness of 2·1 mm. The uterine epithelium (·016–·018 mm. thick) is little advanced,

appearing as a low columnar epithelium with close-set nuclei, plump and rich in chromatin. The uterine glands (·04–·05 mm. in diameter) are well convoluted, luminated throughout, and ciliated. The connective tissue is markedly œdematous in the superficial half of the mucosa, and particularly rich in leucocytes; in the deeper part of the same, between the basal coils of the glands, it is more cellular. Below the uterine epithelium there is the usual compact zone of connective tissue, which is rich in leucocytes and fairly vascular.

Case 2 (No. 13, 25 . vii . '02).—Female killed four days after final copulation, with 1 and 2-celled ova and abnormals in the uteri. The uterus is essentially similar to that of Case 1. Mitoses in the uterine and glandular epithelium are not infrequent.

The Nursing Period.

Parturition in mammals is followed by a period termed by Heape (11) the "nursing period," during which the young are nourished by the milk secreted in the mammary glands of the mother. As is known, there is a noteworthy difference in the way in which the milk is obtained by the young in the Eutheria and Metatheria. In the former the young are perfectly free and seek the teat of the mother, from which they suck the milk. The new-born marsupial, on the other hand, which is brought forth in a much less advanced condition than the Eutherian, is transferred to the pouch, presumably by the mother with the aid of her lips, and becoming fast to a teat, the milk is pumped down its throat.

The nursing period in the marsupial falls into two distinct phases :

(1) A period of fixation, during which the lips of the young one are fused in such a manner that it is firmly attached to the teat, which it cannot leave. During this period the milk is said to be forced periodically into its mouth by the contraction of the muscles of the pouch area of the parent. This period in Dasyurus lasts from seven to eight weeks (13).

(2) A free period, during which the lips of the young animal are no longer fused, so that it can leave the teat at will, but is still entirely dependent on its mother for food. In Dasyurus, this period extends over eight or nine weeks. The total time occupied by these two periods, which together form the true nursing period, is about four months in Dasyurus (**13**).

After this period, the young move about freely away from the mother, and begin to eat, although they still may make occasional use of the pouch.

This marks the conclusion of the reproductive cycle, and the various organs now return to an ancestrous condition, or state of rest, until the following year.

PSEUDO-PREGNANCY.

Ovulation is followed by the formation of corpora lutea. According to Ancel and Bouin (**1**), in animals in which ovulation is spontaneous, two distinct kinds of corpora lutea are formed; (*a*) in the pregnant animal, a gestative corpus luteum (corpus luteum verum), which persists for a long time, and (*b*), in the non-pregnant animal, a periodic corpus luteum (corpus luteum spurium), which is not so fully developed as the former, and has only a transitory existence. No such distinction is possible in Dasyurus, for the structure of the corpus luteum is identical, whether pregnancy follows ovulation or not, and there is evidence to show that in the non-pregnant animal the corpus luteum persists for a considerable time, some weeks at least, and even then shows no sign of degeneration (vide **21**).

The pouch continues to enlarge after ovulation, and the sweat and sebaceous glands of the pouch area reach a state of development and activity comparable to that attained in the pregnant animal. In some cases, it was observed that the female started to clean out the pouch in the same way as does the pregnant female, in preparation for the reception of the young.

The most striking changes during this period, however, occur in the mammary glands. These changes have already been fully described (20), so that here it is only necessary to emphasise the fact that the glands hypertrophy in precisely the same way as do those of the pregnant animal, and ultimately reach a state of development, which is at least as advanced as that in a female thirty-six hours after the birth of the young.

Uterine Changes.

Case 1 (31 . v . '01).—This female died in captivity the day after its arrival. The uteri were slightly enlarged, but no ova were found. Examination of the ovaries reveals the presence of corpora lutea in the last stages of growth, there being present in their central region small spaces not yet filled either by lutein cells or by connective tissue. Ovulation must have occurred some days previously.

The uterine mucosa measures 2–3 mm. in maximum thickness. The uterine epithelium (·027 mm. thick) is formed of narrow columnar cells with closely packed small nuclei, ovalish or rounded in form, occupying the mid-region of the layer. The uterine glands appear crowded together and are markedly convoluted. The connective tissue is compact below the epithelium and fairly so between the glands. The mucosa is not specially vascular.

This case is of importance as showing the condition of the uterus in a female some time after ovulation and before the onset of the degenerative and regenerative changes in the mucosa.

Our next two cases are of special interest as showing stages in these degenerative and regenerative changes in the non-pregnant uterus after ovulation, changes which we are convinced are the homologues of those seen in the normal post-partum uterus.

Of Cases 2 and 3 we have no further record beyond the statement that the uteri were opened but nothing was found.

Case 2 (vii. 06) Pl. 7, fig. 6. Corpora lutea are present in the ovaries. They are at a stage towards the end of the growth period, and in their degree of development are intermediate between those of Case 1 and Case 3.

The mucosa has a maximum thickness of 2·9 mm. The uterine epithelium appears as a layer of quite low cubical cells (·006—·009 mm. in thickness), which essentially resembles that of a uterus seven days post-partum. Vacuolar spaces occur here and there in the epithelium, but the nuclei on the whole are plump and rich in chromatin and show no obvious signs of degeneration. It is therefore probable that the uterine epithelium has already been reconstituted.

The uterine glands are in a very interesting condition, the appearances presented strikingly recalling those seen in the glands of post-partum uteri. Over the major portion of the thickened area of the mucosa, the basal portions of the glands are markedly convoluted and greatly hypertrophied but show no signs of degenerative change. They average in diameter ·072 mm. (varying from ·056—·1 mm.), and are lined by an epithelium composed of very narrow, high columnar cells (·024—·04 mm. in height), with small basally situated nuclei. The gland lumina are distinct, but small, and in many cases contain a homogeneous secretion which stains deeply with esoin. The convolutions of the glands are very compactly arranged, with little or no connective tissue between them.

Comparison of these hypertrophied glands with those of a pregnant uterus with blastocysts 1·5 mm. in diameter shows that whilst the gland convolutions in the latter are not so compactly arranged, the glands themselves are slightly thicker (·08—·096 mm. in diameter, epithelium ·032 mm.) and the gland lumina are much larger.

In the more superficial region of the thickened area of the mucosa, as well as round its periphery, the glands and the portions of them situated therein present a very different appearance. They are much less convoluted and much less compactly arranged. Moreover, instead of possessing thick glandular walls and small lumina, they are lined by an

epithelium of the quite low cubical type ($\cdot$006—$\cdot$015 mm. in thickness) and possess large and distinct lumina. These latter are not empty, but contain either a homogeneous coagulum in which cells may be embedded, or, as is more often the case, they are occupied by a more or less compact mass of cells (Pl. 7, fig. 9). The cells possess large, rounded or polygonal cell-bodies which stain deeply with eosin, and nuclei which are occasionally found in division and which are quite similar to the nuclei of the surrounding epithelial cells.

The transition between these altered portions of the glands and the more deeply situated unaltered or rather hypertrophied parts is somewhat abrupt, so that it is difficult to obtain very definite evidence as to how the intra-luminar cells originate. We are of opinion, however, that they are derived from the hypertrophied gland epithelium by a kind of desquamation process, and that they increase in size after their separation. If that view is correct, then we must regard the epithelium of the thin-walled portions of the glands as being in process of regeneration. In this connection, it is worthy of note that mitoses are not rare in the epithelium, and that the superficial portions of the glands close below the openings into the uterine cavity are ciliated.

The connective tissue is no longer œdematous, but appears as a compact, richly cellular tissue. Leucocytes are not specially abundant, but are most numerous in the more superficial region of the mucosa, where there are also present numbers of capillaries of varying size. It is worthy of note that there are no blood extravasations.

The uteri in this Case, with the uterine epithelium and much of the gland epithelium regenerated, may therefore be described as well on the way towards the resting condition. The uterine epithelium resembles that of the seven days' post-partum uterus, the glands, those of the two days' post-partum uterus.

Case 3 (1900) (Pl. 8, fig. 7).—The ovaries show corpora lutea at the end of the growth period, just older than those of Case 2.

The mucosa has a maximum thickness of about 3·5 mm. and presents a markedly folded surface. The uterine epithelium appears as an irregular folded layer of low columnar cells and shows evident signs of degenerative changes, e.g. irregularities in contour both of its cells and nuclei, occurrence of vacuoles in the cytoplasm, presence of degenerated nuclei and of diffused darkly staining (chromatinic ?) granules. Moreover, blood extravasations from the superficial capillaries would appear to have taken place through clefts formed by the breaking down of the epithelial cells. These extravasations appear to have been slight, but are undoubted. Besides corpuscles, there are present in them, cell remnants and small, darkly staining granules. In this female, accordingly, the uterine epithelium, unlike the preceding case, has not yet been reconstituted.

The uterine glands are in essentially the same condition as those of Case 2. In the deep portion of the central region of the thickened area of the mucosa, the parts of the glands therein situated appear for the most part closely packed and hypertrophied. The gland epithelium is formed by high, columnar, pale-staining cells, often vacuolated, and with small basally situated nuclei. The gland lumina are distinct and in some cases contain a staining coagulum.

Elsewhere the glands are in process of being reconstituted. They are lined by a low flattened to cubical epithelium and their lumina are occupied either by coagulum with embedded cells or by cellular masses, which differ from those of Case 2 only in that the cells are in course of degeneration.

The connective tissue differs strikingly from that of Case 2, being in the form of a fine reticular tissue, œdematous and extremely rich in leucocytes.

Numerous enlarged blood-vessels are present in the mucosa, whilst in actual contact with the under surface of the uterine epithelium there are numbers of fine capillaries such as occur normally in this position in the late pregnant uterus.

Case 4 (31. vii . '99) (Pl. 7, fig. 8.—The record is as follows :

14 . vii . '99.—Female brought in.

15 . vii . '99.—Put with male ; no record of copulation.

17 . vii . '99.—Pouch large, dirty.

20 . vii . '99.—Pouch large, fairly clean, teats red, sebaceous glands just appearing.

22 . vii . '99 ⎫
24 . vii . '99 ⎪ Pouch relaxed, but not quite clean, glands
26 . vii . '99 ⎬   small.
28 . vii . '99 ⎭

31 . vii . '99.—Pouch quite relaxed, moist, glands fairly well marked ; apparently pregnant.

Killed, but nothing found in the uteri, which were enlarged. The ovaries were sectioned and show full-grown corpora lutea, somewhat older than those of Case 3.

The mucosa is thickened and its surface is thrown into well-marked folds. The uterine epithelium is very similar to that of Case 3, and is for the most part in process of degeneration. In places it appears as a practically unaltered layer of cubical cells, but over most of its extent, it exhibits evident signs of degenerative change. It is very irregular both in contour and thickness and is invaded by leucocytes. Moreover, cells may be seen in process of desquamation from its outer surface, and in places, it appears actually to have broken down, since extravasated blood, in which occur degenerate cell-products, is present in small quantities in the uterine cavity close to the surface of the epithelium.

The uterine glands have been reconstituted throughout their extent. They are lined by a quite low epithelium, and their lumina are occupied more or less completely by cellular masses (containing leucocytes), in a more or less advanced stage of degeneration.

The connective tissue is very cellular and is invaded by large numbers of leucocytes.

Making allowance for individual variations due to age or other causes, the foregoing Cases afford evidence of the occurrence of progressive, regressive and regenerative changes in the uterine mucosa, during this period of pseudo-pregnancy.

(a) Progressive Changes.—The mucosa as a whole undergoes marked thickening, its vessels enlarge, and in particular a rich plexus of capillaries is established immediately below the uterine epithelium. The uterine glands hypertrophy, their epithelial cells assume an elongate columnar form and secrete actively.

These changes are identical with those seen in normally pregnant uteri. The only noteworthy difference in the progressive alterations in the pseudo- and normal pregnant uterus concerns the uterine epithelium, the cells of which in the former fail to become transformed into the high columnar elements characteristic of the latter.

(b) Regressive Changes.—The uterine epithelium does not appear to be shed as a whole, but undergoes partial degeneration and desquamation, and it is noteworthy that these degenerative changes may so effect the immediately underlying capillaries as to lead to their rupture and the consequent appearance of extravasated blood in small quantities in the uterine cavity.

We have so far not observed such extravasation in normal post-partum uteri, though it is possible that this may occasionally occur. On the other hand, the very marked extravasations into the connective tissue which are found in post-partum uteri are not met with in the pseudo-pregnant cases.

The uterine gland epithelium also appears to undergo a process of desquamation—at all events cells are shed from it into the gland lumina, where they form cellular masses which eventually degenerate. Comparison of our preparations of pseudo-pregnant uteri with those of normal post-partum uteri demonstrates beyond all possibility of doubt that the regressive changes in the glands are identical in the two. In post-partum uteri, we find the same reduction of the gland epithelium from the high columnar to the low cubical type taking place, and accompanied, as in the pseudo-pregnant uterus, by the appearance in the gland lumina, of masses of cells which eventually degenerate.

(c) Regenerative Changes.—The uterine epithelium

is reconstituted, and the low cubical epithelium of the uterine glands persists like that of the post-partum uterus to form the lining of the resting glands. The connective tissue re-assumes its more compact form, and the mucosa as a whole undergoes marked decrease in thickness and returns to the condition of rest.

It is difficult, indeed, impossible, to ascertain the exact duration of this period of pseudo-pregnancy, but so far as we can judge with the aid of our records, it probably extends over about two weeks. We have, for example, a very full record of a female, apparently in heat about June 28th and killed on July 15th, i. e. seventeen days after heat, and of another female in heat on June 9th (copulation on June 9th to 12th) and killed on June 30th, i. e. twenty-one days after heat. In both, nothing was found in the uteri. In both, the ovaries showed old corpora lutea, the pouch was greatly enlarged the mammary and other glands being well-developed, whilst the uteri were extremely large and vascular (measuring in the first-mentioned female by 4 by 1 cm. and in the second 3·9 by 4·1 by 1·6 cm. in diameter).

Metœstrus.

After the changes described in the preceding section have taken place, the whole of the reproductive organs gradually return to a state of rest. This metœstral period corresponds functionally to the similarly named one in the Eutheria, only there is the striking difference that, whereas in the unimpreg-nated Eutherian mammal, metœstrum follows immediately after ovulation, in the unimpregated marsupial, the two are separated by post-œstrus and pseudo-pregnancy, the two periods together occupying at least a fortnight, and probably longer.

Summary.

Dasyurus is monœstrous and has one breeding season a year, which begins at the end of May or early in June and

lasts into the first fortnight in August (i.e. it extends over the winter months).

The male does not appear to experience a marked rutting season.

Copulation is similar to that of Didelphys (Selenka), and the sperms can remain alive in the Fallopian tubes for at least two weeks.

Anœtrus.—The anœstral period lasts more than half the year.

Pro-œstrus.—Pro-œstrus appears to extend over a varying period of from four to twelve days.

During this time, the lips of the cloaca become swollen, the pouch enlarges somewhat and becomes slightly tumid and moist, and the Graafian follicles increase in size and become vesicular. The uterine mucosa increases in thickness and becomes more vascular, its glands lengthen and become convoluted and the uterine epithelium also tends to thicken.

Œstrus.—Œstrus lasts usually for one or two days and is the period during which copulation occurs.

The changes already initiated during pro-œstrus in the various parts of the reproductive system are continued without interruption.

Post-œstrus.—Post-œstrus, which term we employ to designate the period following œstrus and terminated by ovulation, occupies as a rule about five or six days.

The tumidity of the cloacal lips disappears, but the changes in the pouch and uterus still continue, not, however, very actively.

In the ovary (1) the ova give off the first polar body and the spindle for the second meiotic division is formed.

(2) The follicles attain maturity and ultimately rupture, setting free the ova.

Ovulation.—Ovulation marks the end of this period and occurs generally about five or six days after œstrus. It is spontaneous and independent of copulation and is remarkable because of the large number of ova liberated. Ovulation is succeeded (a) by pregnancy or (b) by pseudo-pregnancy.

Pregnancy.—Fertilisation is effected in the upper part of the Fallopian tube and the second polar body is there given off.

As a rule more young are born than can possibly survive owing to the limited accommodation in the pouch.

The gestation period is not less than eight and not more than fourteen days, but the interval between copulation and birth is usually considerably longer.

Corpora lutea are formed and persist throughout the greater part of the time that the animal is lactating.

Nursing Period.—This period may be divided into two phases.

(1) Period of Fixation.—A period, lasting for seven or eight weeks, during which the young are firmly attached to the teats.

(2) Free Period.—A period of eight or nine weeks when the young are free in the pouch but dependent on the mother for food.

After this time the various organs gradually return to a state of rest.

Pseudo-pregnancy.—We have applied this term to the period following ovulation in cases where the ova have failed to develop, because of the occurrence in it of a series of changes in the reproductive organs essentially identical with those met with in pregnant females.

Corpora lutea are formed in the ovaries which are identical with those in the pregnant female.

The pouch enlarges, and its sweat and sebaceous glands reach a state of hypertrophy and functional activity comparable to that in the pregnant female.

The mammary glands also enlarge and reach a state of development equal to that in a female thirty-six hours after parturition.

The uteri enlarge and become vascular, often to a marked degree.

The uterine mucosa undergoes a series of changes, progressive, regressive and regenerative.

THE ŒSTRAL CYCLE IN THE NON-PREGNANT FEMALE DASYURUS.

	General symptoms.	Uterine changes.	Ovarian changes.
ANŒSTRUS . .	Rest.	Rest.	Rest.
PRO-ŒSTRUS .	Swelling of cloacal lips. Pouch commences to become tumid.	Commencement of constructive stage. Hypertrophy of mucosa with increase of vascularity. Proliferation in uterine epithelium and glands.	Growth of follicle and contained ovum.
ŒSTRUS . .	Period of desire.	Continuance of above.	Continuance of above.
POST-ŒSTRUS .	Pouch tumid and slightly moist, growth of sweat and sebaceous glands of pouch area. Regression of cloacal swelling.	Continuance of above but not very actively.	Follicles attain maturity. Separation of first polar body and formation of second polar spindle. Ovulation.
PSEUDO-PREGNANCY .	Further enlargement of pouch accompanied by growth of mammary glands as in pregnancy.	Continuance of above. (a) Progressive changes in uterine glands especially. (b) Regressive (destructive) changes, e.g. degeneration and desquamation of uterine and gland epithelium. Extravasation of blood. (c) Regenerative (repair) changes. Recuperation of mucosa.	Formation and growth of corpus luteum as in pregnancy.
METŒSTRUS . .	Return to rest.	Return to rest.	Return to rest.

ANŒSTRUS.—As Dasyurus is monoestrous, metœstrus is followed by anœstrus, which lasts until the following year.

THE ŒSTRAL CYCLE IN THE NON-PREGNANT FEMALE EUTHERIAN.

	General symptoms.	Uterine changes.	Ovarian changes.
ANŒSTRUS	Rest.	Rest.	Rest.
PRO-ŒSTRUS	Swelling and flushing of vagina. General symptoms termed by breeders "coming in heat."	Constructive stage: Swelling of mucosa. Proliferation in uterine epithelium and glands. Hyperæmia and congestion. Destructive stage: Increased hyperæmia. Breakdown of vessels and formation of blood lacunæ.	Growth of Graafian follicle.
	Appearance of menstrual discharge if present.	Rupture of lacunæ, degeneration of uterine and glandular epithelia. Formation of menstrual clot if present.	Maturation of ovum.
ŒSTRUS	Period of desire. Vagina still swollen and flushed.	Stage of repair: Recuperation of various uterine elements.	Discharge of ova (in animals with spontaneous ovulation).
METŒSTRUS	Return to rest.	Return to rest.	Formation of corpus luteum spurium (in animals with spontaneous ovulation).

One of two conditions may then ensue. (1) The various organs may remain for a few days in a stage of comparative inactivity, but almost immediately recommence the changes indicative of Pro-œstrus. This short interval is termed dioestrus, and the animal is said to be Polyœstrous. (2) The various organs may remain for a long time in a state of rest, this being the proper Anœstrus, and the animal is said to be Monœstrous.

Metœstrus.—This is an indefinite period during which the whole of the reproductive organs return to a state of rest.

CONCLUSIONS.

Comparison of the Œstral Cycles in the Metatheria and Eutheria.

A comparison of the tables of the œstral cycle in the non-pregnant Dasyurus and in the non-pregnant Eutherian (pp. 163–164), following the schemes of Heape (**11**) and Marshall (**18**), discloses certain rather striking and important differences between them. These differences are seen at a glance in the

SCHEME OF REPRODUCTIVE CYCLE IN—

(1) DASYURUS.	(2) EUTHERIAN.
Anœstrus.	Anœstrus.
Pro-œstrus.	Pro-œstrus (uterine degeneration).
Œstrus.	Œstrus (ovulation). Diœstrus.[1]
Post-œstrus (ovulation).	Metœstrus.
Pregnancy. Pseudo-pregnancy (uterine degeneration).	Pregnancy.
Nursing period. Metœstrus.	Nursing period.
Anœstrus.	Anœstrus.
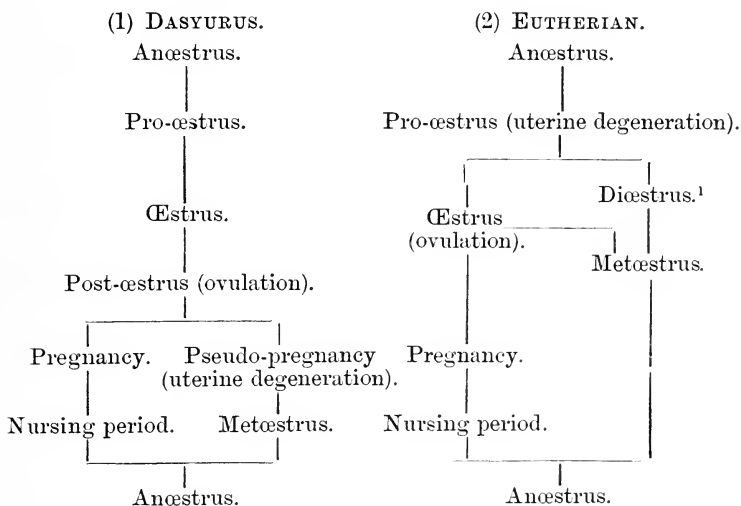
[1] This period only occurs in polyœstrous animals.

scheme which we have drawn up of the reproductive cycle in the Marsupial (as represented by Dasyurus), and what may be taken to be the usual cycle in the Eutherian mammal as set forth by Heape and Marshall. They concern more especially the time-relations of the degenerative uterine changes

and of ovulation to the cycle as a whole. Thus, taking œstrus as an easily recognisable and obviously equivalent event in the two cycles, we see that, whilst in the Marsupial, ovulation only occurs at an interval of some days after œstrus, in the Eutherian, ovulation either coincides with, or follows immediately after œstrus. We have emphasised this important difference by applying the term "post-œstrus" to the period intervening between œstrus and ovulation.

Furthermore, there is a very striking discrepancy in the time of occurrence of the degenerative changes in the uterine mucosa in the two. In the marsupial, these succeed ovulation; in the Eutherian they not only precede ovulation, but also œstrus. This, we regard as the most striking difference in the two cycles.

In the preceding pages we have produced evidence demonstrative of the essential similarity in Dasyurus of these degenerative changes in the pseudo-pregnant and post-partum uteri. Indeed, comparison of the entire series of changes in the organs directly concerned with and related to reproduction in females which have ovulated and have failed to become pregnant, and which we have already described (ante, pp. 21–28), with those seen in the same parts of the pregnant female, entirely justifies us in recognising the occurrence of what we have termed a period of pseudo-pregnancy in the œstral cycle of the marsupial and in affirming that that period represents the phase of true pregnancy.

That being so, the question arises, What part of the Eutherian cycle corresponds to pseudo-pregnancy in the marsupial? There can be little doubt but that the uterine degenerative changes seen during the pseudo-pregnancy period in the marsupial are equivalent to those which take place in the Eutherian uterus during pro-œstrus, a conclusion which we think, in view of the evidence herein presented, will meet with general acceptance.

These pro-œstral changes in the Eutherian uterus condition the appearance in some members of the sub-class (e.g. Primates) of a sanguineous discharge—the menstrual

flow.   This latter we regard as the morphological and physiological equivalent of the degenerated epithelial elements and blood extravasations met with in the pseudo-pregnant uterus of the marsupial.[1]   We are therefore in complete agreement with those writers (Van Herwerden (**12**), Grosser (**7**), Hitschmann and Adler (**15**), Beard (**3**) and others) who hold that " menstruation   .   .   .   is the expression of changes in the uterine mucous membrane which are associated with preparations for the reception of a fertilised ovum," and that " menstruation itself is only a secondary process—a degeneration of the mucous membrane which from a failure of pregnancy has not been able to fulfil its purpose " (Grosser, loc. cit., pp. 97 and 102).

Our observations afford no support for the view that "menstruation is identical with ʻheatʼ" (Heape (**11**) p. 59), nor for the view " that menstruation in the Primates is the physiological homologue of the pro-œstrum in the lower mammalia" (Marshall (**18**) p. 162).

As is generally recognised, menstruation is simply the outcome of degenerative uterine changes, and although these are manifested at the end of the pro-œstral period in Eutheria, our observations demonstrate that in the marsupial, they succeed both œstrus and ovulation, and cannot therefore be considered as forming any part of the pro-œstral phase.

Now, bearing in mind the lowly position which the Marsupials occupy in the mammalian series, and taking into

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[1] Wiltshire states (**27**, p. 397), on the authority of Bartlett, that in kangaroos in the gardens of the Zoological Society, " a mattery, slimy " discharge from the cloaca, slightly tinged with a reddish colour, had been observed by the keeper in females at the time of "heat." We have one record of the occurrence of an apparently corresponding discharge from the cloacal aperture in Dasyurus.   The discharge is described in our notes as "a whitish glairy secretion, consisting of refractive granules, round and angular," and is regarded as the secretion of the cloacal glands.   We are not inclined to attach any importance to its occurrence in the present connection, but it is quite possible that the cloacal glands may become more active during proœstrus.

account the close similarity which is apparent between the
cyclical changes in the pregnant and non-pregnant marsupial,
it can hardly be doubted that the œstral cycle in this group
is not only simpler but much more primitive than that of
Eutheria, so far as known. If this be admitted, then it
follows that, in the Eutheria, the inclusion in the pro-œstral
period, of the degenerative uterine changes which condition
menstruation is a purely secondary phenomenon, the result
of a thrusting forward of these events to a much earlier
period in the œstrous cycle as compared with the mar-
supial.

So far, then, as concerns the uterine changes during pseudo-
pregnancy, we conclude that these are represented in the
Eutheria by the alterations which occur in the mucosa during
the latter part of pro-œstrus, and which, in some forms, cul-
minate in menstruation.[1]

What induced this remarkable dislocation of events in the
Eutherian œstrous cycle is a problem by no means easy of solu-
tion, but we venture to suggest that it may perhaps be
brought into relation with the omission or marked shortening
in the Eutheria, of the post-œstral period whereby ovulation is
directly transferred to œstrus.

It is legitimate to assume that the shortening of the cycle
in this way may have induced an increased growth of the
mucosa during the pro-œstral period, and that this growth in
its turn may have directly conditioned the earlier occurrence
of the degenerative and regenerative changes, with the result
that these latter now came into operation before instead of
after the ovulation to which they were primitively related.
Whether or not there be anything in these suggestions, there
can be little doubt that the precocious onset of the degene-

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[1] The histological changes in the premenstrual uterus of the human
subject have been fully described by Hitschmann and Adler in their
important paper (15) published in 1908. These observers demonstrate
that the changes in the premenstrual uterus are identical with those
seen in the early pregnant uterus. To this paper, which contains a
very full bibliography, the reader is referred for further details.

rative changes in the mucosa, followed as they are by regenerative growth, is of the nature of an adaptation, of obvious advantage, as is generally recognised, from the point of view of early placental formation (embedding, trophoblastic attachment, etc.).

Whilst, then, we believe that the uterine changes characteristic of the pseudo-pregnant period in the marsupial have been shifted forwards to pro-œstrus in the Eutheria, it remains to be pointed out that amongst the Eutheria, alterations in the ovary and mammary gland corresponding to those seen in the pseudo-pregnant marsupial have indeed been recognised, but do not appear to have been brought into correlation with the œstral cycle.

Various authors (e. g. Ancel and Bouin (1)) have described the formation of corpora lutea spuria, so-called, which in animals ovulating spontaneously, last for a shorter time than in the pregnant female.

Further, as regards the mammary glands, it has been stated (Frank and Unger (6) and others) that a growth of these occurs during the œstral cycle, and according to Ancel and Bouin (2), only after the formation of corpora lutea. One of us (O'D.) has evidence confirmatory of this latter statement so far as concerns the rabbit.

These changes in the ovary and mammary glands have thus retained their original position in the cycle, i. e. they occur after œstrus and ovulation as in the marsupial.

The Monœstrous and Polyœstrous Conditions.

According to Heape (11), monœstrous mammals are those which experience a single œstrus during each sexual season, i. e. in which the anœstrous cycle alone occurs, whilst polyœstrous mammals are those " whose sexual season is occupied by a series of diœstrous cycles, or in other words, those who experience a series of recurrent œstri " (p. 9). Heape questions "if in the present state of our knowledge it is possible to determine which is the original of these two con-

ditions" (Heape, loc. cit., p. 18). We propose here to
consider this question very briefly in the light of such evidence
as we have of the œstral cycle in the Marsupials and Mono-
tremes, which in respect of their reproductive phenomena
generally (but making exception of the pouch of the Mar-
supials) are undoubtedly more primitive than the Eutheria.

We have already shown earlier in this paper that the œstrous
cycle in Dasyurus occurs only once in the breeding season,
i. e. that Dasyurus is monœstrous. As regards other
Marsupials we have no definite evidence, but our records, and
those of others (Semon (**25**) Caldwell (**5**)), indicate that
Trichosurus, Phascolarctus and Phascolomys breed but
once a year, each species having its own particular breeding
time, regularly recurring. We think in view of the posi-
tive evidence we have concerning Dasyurus, the cautious
statement of Heape (loc. cit., p. 21) that "among certain
wild animals which are known to undergo parturition only
during a very circumscribed time, the monœstrous condition
may be assumed as probable . . . ," may be accepted
and applied to the forms mentioned.

Selenka, however, makes the definite statement in regard
to Didelphys marsupialis (**23**, p. 104)—"Die Brunst der
Weibchen tritt normaler Weise nur ein Mal im Jahre ein.
Wenn aber den Mutterthieren die Jungen kurz nach dem
Gebären aus dem Beutel fortgenommen wurden oder wenn die
Begattung, was öfter vorkam, aus Mangel an Geschicklichkeit
der Männchen nicht gelang, so können die Weibchen 4–6
Wochen später zum zweiten male im Jahre brünstig werden,
spätestens jedoch Anfang Juni."

Further, with reference to Hypsiprymnus cuniculus, he
states (**24**, p. 174)—"Die herrannahende Brunst . . . zu
verschiedenen Jahreszeiten, im Frühling, Herbst und Winter
beobachtet wurde."

Wiltshire, again (**27**, p. 397), states on the authority of
Bartlett that the kangaroos in the Gardens of the Zoological
Society "display sexual excitement in September . . .
and also in our spring month of April."

We have had no experience of Didelphys, but cannot regard Selenka's statement as indubitable in the absence of any record of the condition of the ovaries, whilst as regards Macropus spp., we can only say that our records indicate that in New South Wales at all events they certainly breed during the summer months (December to February). Irrespective, however, of the number of breeding seasons per year, we have no evidence as to whether the macropods are monœstrous or not.

As regards the Monotremes, our own experience confirms the statement of Caldwell (5) and Semon (26)[1] to the effect that the breeding season recurs but once annually. In view of the facts that at most two eggs in the single functional ovary reach maturity at the same time, and that the breeding season is of such short duration that it would appear to be impossible for a second set of eggs to become full-grown within its limits, we consider it justifiable to assume that Monotremes are, like Dasyurus, monœstrous.

In view of these considerations and of others relative to the breeding habits of reptiles which we do not think it necessary to bring forward here, and in view, moreover, of the lowly position occupied by the monotremes and marsupials in the mammalian series, we find it difficult to avoid the conclusion that the monœstrous condition is the primitive one, and that the polyœstrous condition has been secondarily derived from it. This latter condition where it occurs amongst the Eutheria is obviously advantageous, since it permits of the production of a larger number of young, or at least provides greater opportunity for successful impregnation.

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EXPLANATION OF PLATES 6, 7 AND 8.

Illustrating Prof. J. P. Hill and Dr. Chas. H. O'Donoghue's
paper on "The Reproductive Cycle in the Marsupial,
Dasyurus viverrinus."

[All the photo-micrographs are from uteri of Dasyurus and are taken
at a magnification of 30 diameters except figs. 9 and 10, the magnifica-
tion of which is 250 diameters.]

PLATE 6.

Fig. 1.—Uterus, pro-œstral Case 1 (17 . vi . '99).

Fig. 2.—Uterus, pro-œstral Case 2 (2 . 21 . v . '03).

Fig. 4.—Uterus, post-œstral Case 1 (8, 23 . vii . '02).

Fig. 5.—Uterus, early pregnant Case 1 (15, 19 . vii . '01).

PLATE 7.

Fig. 3.—Uterus, œstral Case 1 (12 . vi . '02).

Fig. 6.—Uterus, pseudo-pregnant Case 2 (vii . '06).

Fig. 8.—Uterus, pseudo-pregnant Case 4 (31 . vii . '99).

Fig. 9.—Portion of mucosa of same uterus as fig. 6, showing uterine
glands lined by low cubical epithelium and with their lumina filled by
masses of cells.

PLATE 8.

Fig. 7.—Uterus of pseudo-pregnant Case 3 (1900).

Fig. 10.—Portion of a uterine gland from the same uterus as fig. 3,
showing the ciliated gland epithelium.

Further Observations on the Intestinal Trypano-plasmas of Fishes, with a Note on the Division of Trypanoplasma cyprini in the Crop of a Leech.

By

C. H. Martin, M.A.

With Plates 9 and 10 and 2 Text-figs.

Contents.

I. General Introduction.

In a previous paper in this journal ('Quart. Journ. Micr. Sci.,' vol. 55, 1910) I have given a fairly detailed account of the morphology and method of division of the normal active form of Trypanoplasma congri.

In attempting to unravel the rest of the life-cycle of this animal, I thought that it might be of some assistance if I could obtain material of the two other Trypanoplasmas which had been described from the gut of fishes, more particularly

as up to the present no very detailed account has appeared of these forms.

During the summer of 1910 I was able, while occupying the British Association table at Naples, to obtain a number of Box boops, the stomachs of which were heavily infected with a flagellate which I must regard as identical with the form described by Leger as Trypanoplasma intestinalis.

The most interesting point, however, in connection with my material, is that in life this parasite seemed to me always to possess three anterior flagella, though in stained forms they were not usually easy to unravel. For this reason I have decided to re-name this form Trypanoplasmoides intestinalis. I also found the curious form which Leger was inclined to describe as a female Trypanoplasma, and which Alexeieff has more recently described under the name of Trichomonas Legeri. I am inclined to regard this form, for reasons which I hope to give in a later paper, as the zygote of Trypanoplasmoides intestinalis.

My opportunity of working on the parasite in the stomach of Cyclopterus lumpus arose during a stay at the Scottish Fishery Laboratory at Aberdeen, and I should like to take this opportunity of thanking Dr. Williamson for his ever-ready help in connection with this part of the work.

In this case, again, I am not inclined to regard this parasite as a true Trypanoplasma, and for the reasons given below I have decided to retain for this animal Apstein's name of Heteromita dahlii.

I have not succeeded in finding any very important stages in the life-cycle of Trypanoplasma congri, but I have decided to publish here a short account of some curious rounding-up stages, and also of some abnormal division forms. In a future paper I hope to return to some interesting stages of the parasite in Cyclopterus as well as the forms which I regard as the zygote of Trypanoplasmoides intestinalis.

I should like to take this opportunity of thanking the staff

at the Aquarium at Naples for their kindness and help during my stay there, and I should also like to thank Mr. Elmhirst, the Director of the Marine Biological Station, for the great help he gave me in my work at Millport.

Finally, I should like to thank Miss Robertson for her great kindness in giving me some preparations of the crops of leeches containing dividing Trypanoplasma cyprini, and thus enabling me to form some standard from which it was possible to judge how far these various intestinal flagellates of fishes are removed from the true blood Trypanoplasmas. I should like, also, to thank Professor Minchin for his kindness in allowing me to write up the results of this work in his department at the Lister.

II. Methods.

The methods adopted in this paper were the same as those detailed in my previous account of the division of Trypanoplasma congri, but in addition to the stains mentioned there I used Apathy's hæmatoxylin, followed by his Picro-ammoniak fuchsin.

III. Trypanoplasma congri.

I have decided in this part of my paper to give a text-figure of the stages that I have found of the division of Trypanoplasma congri, partly for the sake of comparison with the division stages of the other flagellates from the stomachs of fishes figured below, and partly to elucidate some abnormal division stages which I have found in Trypanoplasma congri. The normal stages of division of Trypanoplasma congri are shown in Text-fig. 1. It will be seen from this that—

(1) The basal granule divides. This is followed immediately by a splitting of the anterior flagellum, and later by the splitting of the posterior flagellum and membrane.

(2) The trophonucleus in the first stage enlarges, the intranuclear chromatin condensing on the karyosome. The trophonucleus assumes first a spindle and later a dumb-bell

TEXT-FIG. 1.
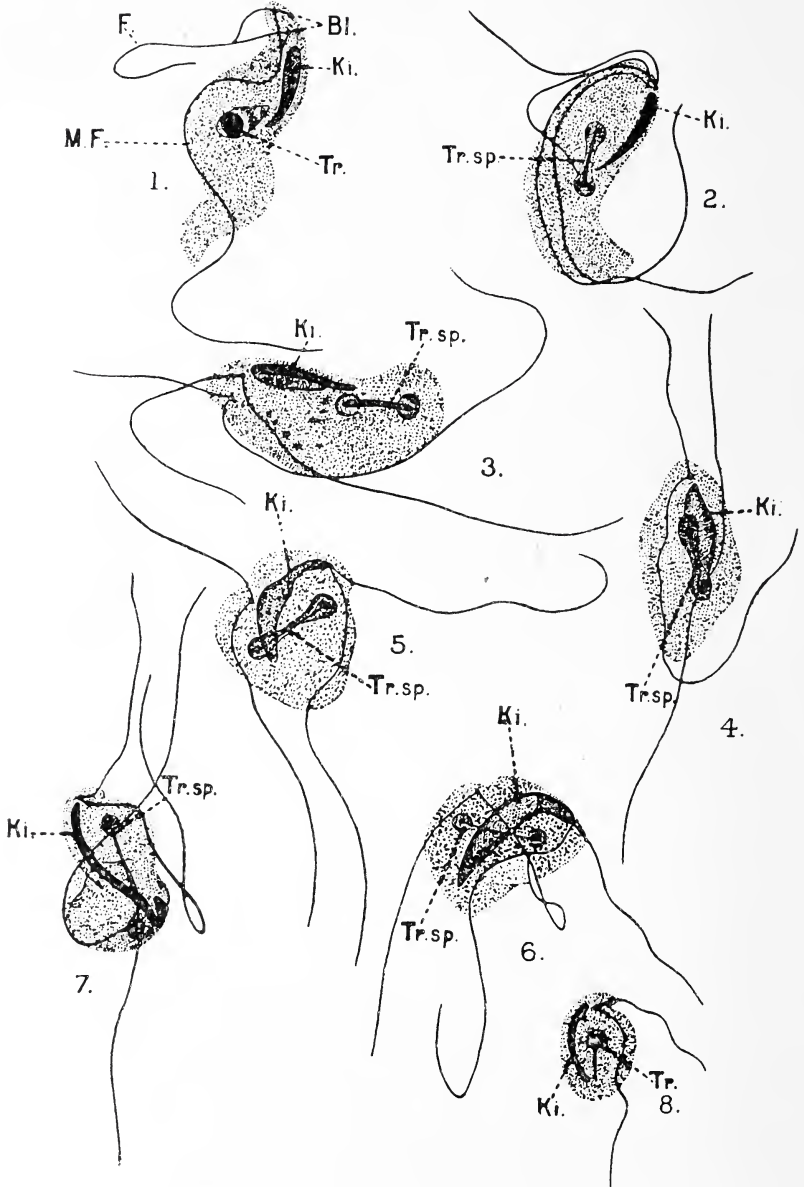
shape, which persists to quite a late stage in division. The karyosome appears to act as an internal division centre, and no trace of individual chromosomes can be seen at any stage of division.

TEXT-FIGURE 1.

Bl. Blepharoplast. F. Free flagellum. Ki. Kinetonucleus. M. F. Membrane flagellum. Tr. Trophonucleus. Tr. sp. Trophonucleus spindle.

Fig. 1.—Early stage of division of Trypanoplasma congri. The whole body of the animal is shorter and stouter. The basal granule has divided, the anterior flagellum is split along about a quarter of its length, and the beginning of the splitting of the posterior flagellum is shown. The kinetonucleus is slightly thicker, and the trophonucleus is distinctly enlarged. The intra-nuclear chromatin granules have probably condensed upon the karyosome, which no longer presents the hard outline characteristic of the resting nucleus.

Fig. 2.—The flagella have now split along their whole length. The karyosome has become drawn out into the characteristic dumb-bell shape within the nuclear membrane.

Fig. 3.—The body of the animal has become still shorter. The kinetonucleus is becoming enlarged and losing its intense capacity for nuclear stain. The dividing trophonucleus is almost parallel to the longitudinal axis of the animal's body.

Fig. 4.—The body of the animal has become still more deformed. The basal granules with their flagella have shifted apart. The kineto-nucleus has become thickened, and has now lost its intense capacity for nuclear stains, its lower border is crossed by the trophonuclear dumb-bell.

Fig. 5.—The basal granules with their flagella now lie at opposite sides of the dividing animal. The lower limb of the enlarged kineto-nucleus has adopted its characteristic position at right angles to the trophonuclear dumb-bell.

Fig. 6.—A slightly later stage than the previous figure, showing the characteristic relations of the enlarged kinetonucleus and the tropho-nuclear dumb-bell.

Fig. 7.—A late stage of division. The two products of division are still united by a broad band of cytoplasm, through which the kineto-nucleus and trophonuclei still retain their connection.

Fig. 8.—A recently divided form showing the characteristic rounded shape, the elongate kinetonucleus, and the unabsorbed strand which had connected the trophonuclei. The full length of the flagella are not shown.

(3) The kinetonucleus increases in length and divides by a simple transverse constriction.

In some rare cases of division it would appear that part of the cytoplasm, with part of the kinetonucleus, is split off, leaving the trophonucleus spindle in the other individual (Pl. 9, fig. 1). As a result of this very rare form of division forms have been found containing only a kinetonucleus. These, I believe, degenerate. The other individual in this division gives rise, I believe, to rather large forms with two trophonuclei (Pl. 9, fig. 2). I have not been able to discover what the final fate of these forms is.

In addition to the abnormal division forms described above I found some stages of a curious process of rounding-up which seems comparable with that found in some Trypanosomes. In the early stage of this process the Trypanoplasmas assume a characteristic head-to-tail position, the anterior and posterior ends being closely approximated. The membrane flagellum now becomes loosened, at first near its anterior end, and this loosening seems then to travel back from that part to the animal's posterior end. At the same time the approximated body-walls on the inner side disappear, so that in the next stage (Pl. 9, fig. 3) the body of the Trypanoplasma is, roughly, apple-shaped. In this stage the two flagella arise together from a point near the anterior pole, both flagella gradually shorten, and in the still later stages both the flagella have disappeared. The nuclei now begin to undergo a series of changes, the significance of which is by no means clear. The kinetonucleus, which originally showed a bunched, compact appearance, becomes a strand-like structure, and at the same time an elliptical vacuole appears in its neighbourhood. The vacuole increases in size, so that it includes the twisted strands of the kinetonucleus, which at first form a darkly staining axis to the vacuole, but later break up into rod-like masses of chromatin.

The trophonucleus appears to become slightly enlarged in the intermediate stages of this process, but the final form of the Trypanoplasma appears to be a spherical animal with no trace

of flagella, and with a large, rounded, vacuolar kinetonucleus and a small trophonucleus. Whether this process may be regarded as the commencement of encystation I am not at present clear.

IV. HETEROMITA DAHLII (APSTEIN); TRYPANOPLASMA VENTRI-
COLI (KEYSSELITZ); DIPLOMASTIX DAHLII (MOBIUS).

This form appears to have had a remarkably chequered history. It was first discovered by Dahl on March 31st, 1887, and a short account of it was given by Mobius in his " Bruchstucke einer Infusorien Fauna der Kieler Bucht " under the name of Diplomastix dahlii. He described it as a spindle-shaped animal with a flagellum double as long as the body at either pole. The next mention of this flagellate that I can find is in Keysselitz's paper on " Generation und Wirts-wechsel von Trypanoplasma Borreli" (p. 37). Keysselitz apparently had overlooked Mobius's work, since he calls the animal Trypanoplasma ventriculi. He gives three figures of the animal under this name, and points out that the blepharoplast is very frequently split into two pieces. Apstein, in his very valuable paper on Cyclopterus lumpus, again gives a short description of this flagellate under the name of Heteromita dahlii, and points out that the name Diplomastix is untenable since the flagella both arise from the anterior pole.

Out of 101 Cyclopterus which Apstein examined he found that 98 were infected. He described the movements of the active forms in some detail, and also some rounded forms which he regards as cysts. He did not succeed in finding any dividing forms, and he draws attention to an interesting mould which is found in association with the flagellates on the stomach-wall. As this animal shows so many points of difference from any other flagellate, free-living or parasitic, of which I have been able to find a description, I have decided to retain for it Apstein's name Heteromita dahlii. In life the animal swims actively, keeping its posterior

flagellum in contact with the body-wall, although there is no evidence of a membrane. Along the line of attachment of the posterior flagellum to the body a number of bright granules can be seen according in appearance with those found in Trypanoplasma. At the posterior end of the body a number of large food-vacuoles are to be seen. The animal very often becomes fixed by the posterior end, and well-marked euglenoid movements are shown, both during progression and when stationary. A mouth can be readily made out in life near the base of the anterior flagellum.

In stained preparations the body of Heteromita dahlii is a spindle-shaped structure, slightly elongated at the anterior end, at which a small cytostome can be seen. The two flagella take their origin near the anterior end from two small blepharoplast granules lying anterior to the kineto-nucleus. The anterior flagellum is much thickened at its base. The posterior flagellum runs back closely attached to the body of the animal, to end freely, and there is no trace of an undulating membrane. Near the anterior end there is an elongated, darkly staining kinetonucleus; this, as Keysselitz has already pointed out, is very variable in shape, but most frequently it seems to consist of a small, rounded anterior portion connected by a narrow neck with a rod-shaped mass (Pl. 9, fig. 8), but forms are met with in which the kinetonucleus presents quite a different appearance (Pl. 9, fig. 7), and in some cases the process of division of the kinetonucleus is carried still further. The trophonucleus is small and spherical. It contains a large karyosome, but most of the chromatin seems to appear in the form of granules lying between the karyosome and the nuclear membrane. In the earliest stage of division which I have found the body of the animal becomes very rounded (Pl. 9, fig. 8), the flagella have already split along their whole length, and the karyosome within the membrane of the trophonucleus has become elongated. The kineto-nucleus is thickened, and shows a slight split at its anterior end. In the next stage one blepharoplast with its attached flagella has travelled back along the animal's body, together

with part of the kinetonucleus, which is apparently just breaking through (Pl. 9, fig. 10). In a still later stage (Pl. 9, fig. 11), the animal has become distinctly pear-shaped, and the kinetonuclei lie so that their longitudinal axes are almost at right angles to each other. The trophonucleus spindle during the later stages of division becomes much elongated, and, as is shown in Text-fig. 2, apparently persists right up to the very last stage of division.

As regards the distribution of the parasite, as far as I can discover it is limited in its active form to the stomach. The

TEXT-FIG. 2.
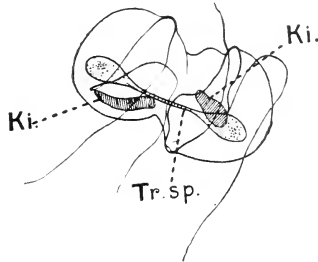
Last stage of division in Heteromita dahlii. Comp. oc. 12 + 1·5 mm. apo. *Ki.* Kinetonucleus. *Tr. sp.* Trophonuclear spindle.

stomachs of the Cyclopterus which I examined give a slightly acid reaction with neutral red. According to Biedermann in Winterstein's 'Handbuch der Vergleichenden Physiologie,' Bd. ii, Jena, 1911, van Herwerden found in Cyclopterus lumpus that "10 c.cm. des filtrierten Magensaftes vom erstgenannten Fische wurden schon neutralisiert von 0·4 c.cm. n/10 NaOH, woraus sich eine Aciditat von nur 0·014 Proz. (als HCl) berechnet."

In order to examine as far as possible the effect on the flagellates of the passage from the stomach into the intestine of its host, I mounted a live smear of the parasite in intestinal juice. The parasites seemed at first to become swollen and revolve round and round instead of exhibiting their usual normal progressive movement. Ten minutes after mounting

a number of granular forms were seen, and half-an-hour afterwards nearly all the parasites were motionless.

Apstein, in his paper referred to above, has paid some attention to the physiology of this form. He states on page 11 of this paper : " Handelt es sich in unserem Falle um parasitismus, oder spielen die Flagellaten vielleicht eine wichtige physiologische Rolle ? Ich mochte das letzere glauben. Das regelmassige vorkommen wurde allein nicht dafur sprechen Die Wasseraufnahme aber hat vielleicht den Zweck, den Magensaft des Seehasen so zu verdünnen, dass die Flagellaten im Magen leben können, andererseits sie verdaut werden würden Welchen Nutzen die Flagellaten aber fur die Seehasen haben, vermag ich nicht anzugeben."

Under these circumstances I thought it might be of some interest to compare the length of life of the flagellate after the death of its host on a piece of stomach placed in sea-water with that on the stomach-wall of the dead animal. The experiment was not very convincing, however, as the mucus on the inner surface of the stomach-wall seemed to be coagulated to a certain extent on placing it in sea-water ; still, forty-eight hours after the death of the host a few active forms were still found in the piece of stomach placed in sea-water, and quite a large number were found on the stomach-wall of the host. In the intestine of the Cyclopterus no trace of the parasite could be found. In the gall-bladder a very small Trichomastix was found, and the rectum was crowded with bacteria and a very large spirochæta.

V.

Trypanoplasmoides intestinalis (Leger) ; Trypanoplasma intestinalis (Leger) ; Cryptobia intestinalis (Alexeieff).

This animal was first described by Leger, without illustrations, under the name of Trypanoplasma intestinalis in 1905 (' C.R. Soc. Biol.,' t. 58) Leger described this parasite as

a true Trypanoplasma. He also drew attention to the presence of a form with three flagella characterised by very curious movements which he was inclined to regard as a female Trypanoplasma.

During the month of July, in 1910, I had the opportunity during a stay at Naples of examining nine individuals of Box-boops, all of which were heavily infected with this flagellate. I was immediately struck, in the examination of the live individuals, by the fact that all of them when carefully examined showed the presence of three free anterior flagella. After the publication of Alexeieff's note I re-examined my stained preparations and found that it was extraordinarily difficult to show the presence of these flagella on the stained forms. During May of 1912 I examined thirteen Box-boops, and again convinced myself of the existence of these three anterior flagella in living forms of this animal. Upon this occasion I also met with the large amœboid form which Alexeieff has called Trichomonas Legeri. I have found some evidence which I hope to publish in a later note pointing to the fact that this form is really the zygote of Trypanoplasmoides intestinalis. In a more recent paper, " Sur la revision du genre Bodo Ehrhg." ('Arch. fur Protistenkunde,' Bd. xxvi, 1912), Alexeieff has re-named the normal form of this flagellate Cryptobia intestinalis. Taking into consideration the relations of the flagella in this form, I have decided to re-name the animal Trypanoplasmoides intestinalis.

In life Trypanoplasmoides is a roughly carrot-shaped organism with a rather blunt anterior end, from which three free flagella take their origin, while a fourth flagellum turns back along the body in connection with the undulating membrane to end freely at the posterior end. The three anterior free flagella are relatively easily seen in living forms, but in stained forms they are usually very difficult to make out, as they are very fine and are generally twisted together in a single strand. The animals in life move in rather a hesitating manner, in a way that is far more suggestive of a

Trichomastix than of a true Trypanoplasma. As the
animal passes forward its anterior end rotates in a small
circle from right to left, like the hands of a watch. The
number of anterior flagella can best be seen in forms
which are anchored by their posterior end; the flagella
then strike upwards individually and swing back together.
As regards the distribution of the parasites in the gut, it is
interesting to note that in the cases I have examined the
stomach was heavily infected as low down as the Pylorus.
Below this no trace of Trypanoplasmoides could be found.
The next portion of the gut, viz. the pyloric cæca and the
commencement of the intestine, were, in the case of the fishes
I examined, free from all flagellates. The rectum was in most
cases seen to be heavily infected with an Octomitus and the
interesting ciliate Opalina saturnalis, which has been
described by Leger and Duboscq.

In the stained forms the flagella seem to take their origin
from a small basal granule lying in front of the darkly
staining mass of the kinetonucleus. The kinetonucleus is
very variable in shape ; generally it is more or less sausage
shaped, but it is often drawn out in the middle, so that it may
appear as if divided into two or more blocks. Such appear-
ances of the kinetonucleus have often been figured as evidence
of division in this and similar forms, but I think it will be clear
from the description of the division given below that they have
really nothing to do with this process. (Pl. 9, figs. 14, 15).

The trophonucleus is generally rather small and spherical,
and contains a fairly large karyosome. Some granules of
chromatin are usually found on the membrane of the nucleus.
Trypanoplasmoides seem to have a very large range of
variation in size, and it is curious to note that the forms
undergoing division are generally found amongst either the
smaller or the medium sized animals.

The details of the division of Trypanoplasmoides are
rather difficult to make out, particularly as regards the
behaviour of the flagella. In the early stages of division the
blepharoplast divides, and at the same time the undulating

membrane splits.   One blepharoplast, attached to its portion
of the split undulating membrane, and carrying probably two
of the free flagella, moves away from its original position.
At the same time the anterior end of the kinetonucleus splits,
and it would appear from the later stages of division that this
part is carried over in connection with the migrating
blepharoplast to a position on the opposite side of the
animal.   The only thing that is abundantly clear in this
division of the kinetonucleus is that no trace of a mitotic figure
can be found, and it is hard to see what part a mitotic
figure could play in such a division.   During division the
chromatin in the trophonucleus becomes condensed within
the nuclear membrane into a dumb-bell-shaped structure.   In
the later stages the dividing trophonuclei move apart, so that
the handle of the dumb-bell becomes much elongated, and
still persists at a stage (Pl. 9, fig. 20) at which the cytoplasm
at the anterior end is already showing distinct signs of
division.

In the case of Trypanoplasmoides I have also found
rounded forms, which apparently result in the structure
shown in Pl. 9, fig. 21.   I have not any evidence to decide
whether this is a stage of the process of encystation.   I do
not propose in this paper entering into a discussion as to the
origin and meaning of the curious form which has been
named by Alexeieff, Trichomonas legeri, as at present I
have no evidence as to its final fate.   It would appear, how-
ever, from the preparations that I have examined that two
Trypanoplasmoides conjugate, and the trophonuclei fuse
together.   At this point possibly the kinetonuclei break
down, although on this point I am rather doubtful.   A
great part of the chromatin is thrown out into the cytoplasm
of the conjugating individuals, and the animal finally comes
to possess the curious elongated nucleus with its tiny karyo-
some characteristic of this form.   I am not at all clear as to
the behaviour of the flagella during this process, but the
undulating membrane seems to be absorbed at a relatively
early stage of the process, and I believe this is also true of

the free flagella at a slightly later stage. I hope to describe
this process in greater detail in a forthcoming paper.

VI. The Division of Trypanoplasma cyprini in the crop of the Leech.

For the purpose of examining how far the various parasites
described above approximate to the type of the true
trypanoplasmas in the blood of fishes, it is obvious that they
must be compared with the latter in the various stages of
their life-cycle. Unfortunately, not much appears to be
known of the morphology of trypanoplasmas. Keysselitz, in
his paper, "Generations- und Wirtswechsel von Trypano-
plasma borreli, Laveran et Mesnil," published a long
account of the life-cycle of a blood Trypanoplasma, but his
figures of division, owing to the unfortunate adoption of a
dry technique, leave much to be desired. This point has
already been insisted on by Minchin in his paper, "Some
Observations on the Flagellates Parasitic in the Blood of
Freshwater Fishes."

Owing to the kindness of Miss Robertson, I recently had
the opportunity of examining some division stages of
Trypanoplasma cyprini in the crop of a leech. Miss
Robertson has shown in her paper on "The Transmission of
Flagellates living in the Blood of Certain Fresh-water
Fishes" ('Phil. Trans.,' B, vol. ccii.), that—"When first
taken into the crop of the leech, the Trypanoplasmas
become rather broader and bulkier in appearance, and
show a very characteristic flowing kind of motion. After
the first few hours they are frequently to be seen in division;
the last stage of this process is passed through with
great rapidity. On the second day after feeding, slender
forms, somewhat comma-shaped, appear in small numbers ;
they are much less undulating in their movements than the
broader forms. These two types persist side by side for
some time, the slender forms gradually increasing in number;
intermediate forms are also present. After some days the

slender forms move forward into the proboscis-sheath. They may occupy this situation as early as the sixth day after feeding. As time goes on they come to lie there in incredible numbers, and very generally attach themselves to the wall by their anterior end, i. e. the end with the free flagellum." It is obvious that in the case of these preparations we are dealing with a specialised type of division resulting in the production of a more elongated trypanoplasma than the normal blood type (Miss Robertson's so-called "comma" type), which is destined to develop into the elongate proboscis form, the true infective form.

The form of Trypanoplasma which is apparently undergoing division in these preparations is shown in Pl. 10, fig. 22, and the elongated infective stage in Pl. 10, fig. 23.

Unfortunately, in the preparations I have examined the early stages of division are very rare, though the later stages are exceedingly abundant. As far as I have been able to see, the new flagella arise by splitting, but this is at present a somewhat doubtful point. Whatever the origin of the new flagella may be, it is obvious, from Pl. 10, figs. 24 and 25, that one blepharoplast after division travels backwards as the kinetonucleus elongates. The elongated kinetonucleus then divides by means of a simple transverse constriction. The division of the trophonucleus is precisely similar to that which has been previously described for Trypanoplasma congri, and it is interesting to note that here again, as is shown in Pl. 10, fig. 29, the products of division may show for a short time the remains of the strand connecting the dividing trophonuclei.

VII. Conclusions.

It would appear that at present the nomenclature of the bimastigate flagellates is in a state of almost inextricable confusion. This state appears to me to depend largely on two factors—(1) the discovery of a free-living bimastigate form with a kinetonucleus (Prowazekia), and the consequent

doubt as to how many of the original species of the genus Bodo may not turn out to be Prowazekia; (2) the recent attempt made by certain authors, on the assumption that the parasite in the receptaculum seminis of snails is identical with the true Trypanoplasmas, to place the intestinal Trypanoplasmas in the genus Cryptobia.

It appears to me that the first step in surmounting these difficulties must be made by a careful study of the genus Cryptobia, the parasite of the snail.

In Friedrich's original paper clear division stages were shown, and these were all of a type which seems very different from that found in true Trypanoplasmas. It does not seem that the more recent account of the division of this form by Jollos can be regarded as replacing Friedrich's original account, since it seems to me more than probable that many of the stages figured by Jollos do not represent division at all, and in any case his series is very incomplete.

On the whole, it seems safest at present to assume that Cryptobia is not a true Trypanoplasma, though the only distinctive feature appears at present to be the absence of distinct undulating membrane in Cryptobia. It seems probable, however, from Friedrich's paper that other distinguishing features would be found by a careful study of the other stages of this animal.

In this connection it is important to remember that Max Kuhn, who has recently studied these parasites in a very large number of snails, has also expressed some doubts as to how far they can be regarded as true Trypanoplasmas ('Vid Die Trypanoplasmen und deren Verbreitung in einheimische und auslandischen Schnecken,' p. 70):

"Die letztere die den Randfaden einer Undulirende Membran darstellt ist sehr viel lockerer mit dem Korper verbunden wie bei dem Trypanosomen. So dass sich haufig auf gefarbter Praparaten die Randgeissel mehr oder wenig frei zeigt und somanchmal scheint als ob hier ein Vertreter der Gattung Prowazekia vorliegt."

If this view that Cryptobia differs from the true Trypano-

plasma be accepted, there now remains for discussion the question as to which, if any, of the so-called intestinal Trypanoplasmas should be included in the genus Trypanoplasma.

If the figures of the dividing Trypanoplasma cyprini shown in Pl. 10 are compared with the figures of the division of Trypanoplasma congri shown in Text-fig. 1, bearing in mind that the division in the case of Trypanoplasma cyprini is a specialised one resulting in the production of a slender form, it will be obvious that there are many points of similarity. I think that these are probably sufficient to retain provisionally the Conger parasite in the genus Trypanoplasma. The parasite in Cyclopterus, *Heteromita dahlii*, appears to me to be separated from the true Trypanoplasma by many important points of difference. Firstly, the absence of an undulating membrane; secondly, the presence of a mouth and ingestion of food; and thirdly, its method of division. I believe that a more detailed examination of this parasite will lead to further points of difference being discovered, particularly in connection with the process of encystation, but these I must leave for a later paper.

As regards the parasites in the stomach of Box, if my view as to the presence of three free flagella in this form be accepted, it is obvious that we are dealing here with a form which is much more closely allied to Trichomonas than to Trypanoplasma. In this case it will be obvious that the kinetonucleus in Trypanoplasmoides is probably not homologous with the similar structures in the other forms. Apart from the structure of the flagella, there are many other points of difference between Trypanoplasmoides and the true Trypanoplasma, viz. the presence of a distinct cytostome in Trypanoplasmoides, the method of movement, and the method of division.

I should now like to refer shortly to a structure to which it seems to me far too much importance has been attached in recent years—the kinetonucleus. If Jollos's description of the division of this structure were to be accepted this importance would be justified, but I think that from the descriptions of the division of the flagellates given above it

will be admitted that the evidence for Jollos's view of the division of the kinetonucleus by means of a mitotic spindle is by no means conclusive. I believe that as a definition of a kinetonucleus all we are at present justified in stating is that the kinetonucleus is a mass, staining darkly with chromatin stains, found near the base of the flagella in some flagellates. That this mass is necessarily homologous in all flagellates appears to me a doubtful assumption, and I believe that when the intestinal flagellates and the free-living Bodos are more closely examined we may find the kinetonucleus in forms for which it would be very difficult to establish a homology.

Finally, it appears to me that the too ready acceptance of the view that the kinetonucleus is a true nucleus comparable to the trophonucleus has led numerous protozoologists of late years into a region of unjustifiable and unprofitable hypothesis.

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EXPLANATION OF PLATES 9 AND 10.

Illustrating Mr. C. H. Martin's paper, 'Further Observations on the Intestinal Trypanoplasmas of Fishes, with a Note on the Division of Trypanoplasma cyprini in the Crop of a Leech.'

LETTERING.

Bl. Blepharoplast. *F.* Free flagellum. *F.F.* Free flagella. *Ki.* Kinetonucleus. *M.F.* Membrane flagellum. *P.F.* Posterior trailing flagellum. *Tr.* Trophonucleus. *Tr. sp.* Trophonucleus spindle.

PLATE 9.

[Figs. 1–6 and 14–21 inclusive were drawn under Zeiss comp. oc. 18 and 1·5 mm. apochromat. Figs. 7–13 were drawn under Zeiss comp. oc. 18 and 2 mm. apochromat.]

Fig. 1.—Trypanoplasma congri. Abnormal division product containing the entire trophonuclear spindle.

Fig. 2.—Trypanoplasma congri. Abnormal individual with two trophonuclei.

Fig. 3.—Early rounding-up stage of Trypanoplasma congri.

Fig. 4.—Later stage of same process.

Fig. 5.—Still later stage.

Fig. 6.—Final stage of rounding-up in Trypanoplasma congri.

Fig. 7.—Normal active individual Heteromita dahlii.

Fig. 8.—Stage preparatory to division in Heteromita dahlii.

Fig. 9.—Early stage of division in Heteromita dahlii.

Fig. 10.—Later stage of division; one blepharoplast with its attached flagella has travelled back and the division of the kinetonucleus is almost complete.

Fig. 11.—The body of the dividing individual has become pear-shaped and the dividing kinetonuclei are now at angles to each other.

Fig. 12.—Later stage of division showing elongation of the dividing trophonucleus.

Fig. 13.—Later stage of division in Heteromita dahlii.

Fig. 14.—Normal active individual of Trypanoplasmoides intestinalis showing three anterior flagella twined together.

Fig. 15.—Active individual of Trypanoplasmoides intestinalis showing origin of three free anterior flagella. In this specimen the flagellum of the undulating membrane is broken away from the body.

Fig. 16.—Early stage of division in Trypanoplasmoides intestinalis—the blepharoplasts have divided. In this specimen two free flagella were attached to each blepharoplast.

Fig. 17.—Slightly later stage of division. The second blepharoplast is not shown.

Fig. 18.—Later stage of division.

Fig. 19.—Later stage of division. The kinetonuclei are completely separated.

Fig. 20.—Very late stage of division. The trophonuclei are still connected by a fine strand, though the commencement of the cytoplasmic division is shown at the anterior end.

Fig. 21.—Rounded form of Trypanoplasmoides intestinalis.

PLATE 10.

Fig. 22.—Trypanoplasma cyprini. From the crop of a leech.

Fig. 23.—Trypanoplasma cyprini. Elongate Proboscis type, from the crop of a leech.

Fig. 24.—Trypanoplasma cyprini. Early stage of division, showing the division of the blepharoplasts.

Fig. 25.—Early stage of division, showing the division of the kinetonucleus.

Fig. 26.—Slightly later stage.

Fig. 27.—Later stage of division, the band connecting the dividing trophonuclei still present.

Fig. 28.—Late stages of division.

Fig. 29.—Last stage of division of Trypanoplasma cyprini.

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CONTENTS OF No. 234.—New Series.

MEMOIRS:

Notes on the Histriobdellidæ.

By

W. A. Haswell, M.A., D.Sc., F.R.S.,
Challis Professor of Biology, University of Sydney.

With Plates 11—14 and 1 Text-figure.

Introductory.

The publication by Shearer in 1910 of an elaborate account of the anatomy of Histriobdella homari (**9**) has greatly added to our knowledge of that remarkable and interesting animal, which had remained neglected, so far as published work is concerned, since 1884, when Foettinger published his valuable observations on it (**3**).

In 1900 I published (**6**) an account of an allied fresh-water form—Stratiodrilus tasmanicus—which I found in the gill-cavities of freshwater crayfishes in Tasmania. Some years ago I found another member of the same group inhabiting the gill-cavities of Astacopsis serratus in streams at elevations of 2000 to 3000 feet in the Blue Mountains of New South Wales; and I have since found specimens of the same form on crayfishes from creeks of various other parts of the same river-system (the Hawkesbury)—the Cataract River and the Loddon River—from small streams flowing directly into the sea on the coast of Illawarra, at Port Hacking (Waterfall Creek, a branch of the Port Hacking River), creeks running into Middle Harbour, Port Jackson, and at Pitt Water off Broken Bay (mouth of the Hawkesbury). I have also found it in the large crayfishes

of the Murrumbidgee River of the great Murray River system, which extends over all New South Wales with the exception of the narrow strip between the Dividing Range and the coast, over southern Queensland and a good part of Victoria and a small part of South Australia. The range of Stratiodrilus would thus appear to be very extensive. So far, in continental Australia, I have found it only on the spiny crayfish (Astacopsis serratus), never on Chæraps bicarinatus,[1] on specimens of which, from widely sundered localities, I have carefully searched for it.

A re-comparison of Stratiodrilus with Histriobdella with the aid of Shearer's paper and with the help of specimens of the European form,[2] shows that the two, though closely allied, are yet very distinct in a number of points. Stratiodrilus is a distinctly more highly-organised animal, as is shown in the more highly differentiated muscular and nervous systems, and in the presence of the three pairs of cirri with their sensory cilia. The excretory system of the two genera is widely different, as will be pointed out subsequently. Other points of difference are the following: The tentacles in Histriobdella are unjointed; in Stratiodrilus they are all two-jointed. The anterior limbs are non-retractile in Histriobdella; freely retractile in Stratiodrilus. Histriobdella has no definite tail region; in Stratiodrilus this region is sharply marked off from the trunk, and its body-cavity is cut off from that of the latter by a partition. A further difference lies in the position of the mouth, which in Stratiodrilus is further forward than in Histriobdella.

The Australian form is nearly related to the Tasmanian. I

[1] In a useful and suggestive paper ('Proc. Zool. Soc.,' 1912) on the Australian crayfishes, Geoffrey Smith proposes to separate the bicarinate crayfishes from the other smooth crayfishes under the new generic name of Parachæraps.

[2] I found them common in Nephrops norvegicus. I have pleasure in thanking Prof. Cossar Ewart, Dr. J. H. Ashworth, and Prof. J. P. Hill for kind assistance.

propose for the former the name Stratiodrilus novæ-
hollandiæ.   The chief differences are in the jaws and,
perhaps, the excretory system.   The jaws are usually con-
siderably longer in proportion to the size of the head, so that
when retracted they project backwards some distance into
the following or " neck "-segment.   In the minute structure
of the jaws with their teeth there are also some constant
points of difference, which will be referred to later.   There
is also a difference in the form of the chitinous penis as shown
in figs. 20–25.   In S. tasmanicus it has its three basal
processes more widely spread and is thinner at the base than
in S. novæ-hollandiæ.

I prefer to postpone any further attempt to determine the
relationships of the Histriobdellidæ with other groups of
animals until I am in a position to give an adequate account
of the development of Stratiodrilus, for which I have
now obtained a good deal of material.

External Features of Stratiodrilus; Movements.

Stratiodrilus novæ-hollandiæ is of similar size to S.
tasmanicus (about 1 to 1·5 mm. when full grown), and
resembles it closely in both external features and internal
structure.   It consists of a head, which is a little less than a
sixth of the total length, five body-segments, and an imperfectly
segmented tail region.   The first of the body-segments, which
is shorter than the others and bears no cirri, may be dis-
tinguished as the neck-segment.   As in S. tasmanicus, the
second, third and fifth segments bear each a pair of two-
jointed cirri, both of which are provided with non-motile
sensory cilia.   The head bears the same appendages as in
S. tasmanicus, viz. a median tentacle, two pairs of lateral
tentacles and a pair of retractile anterior legs.   The tentacles
are all on the average relatively shorter than in the
Tasmanian form, more especially those of the second lateral
pair.   The fourth segment in both species bears no append-
ages in the female, while in the male it bears the retractile

claspers, which are of essentially the same character in both. The posterior limbs do not differ in any essential feature in the two species, except that the cirrus (figs. 18 and 19) with which it is provided is divided into two segments by means of a joint like the other cirri, the corresponding appendage in S. tasmanicus being undivided.

With a close agreement in structure between the Australian (continental) and Tasmanian forms there is associated a correspondingly close resemblance in movements and general mode of life (see **6**, p. 303). In ordinary locomotion the anterior limbs are usually completely retracted, progression being effected exclusively though the peculiar "walking" movements of the hind limbs. Frequently the animal remains for a long time fixed by means of the hind limbs, the body being either extended flat on the surface, the head groping about with the tentacles and occasionally "grazing" on the substratum by series of rapid movements of the jaws, or assuming a half-erect position with the tentacles flexed towards the dorsal side.

Stratiodrilus is able to walk in a reversed position by means of the surface film of the water. But much more remarkable is its ability to progress in mid water. The movement can hardly be described as swimming : slow locomotion through the water is effected by deliberate alternate movements of the hind limbs, as when the animal is progressing on the surface of a solid object. This was observed only in water clouded with suspended flocculent particles.

Though the usual habitat of Stratiodrilus is the gill-cavity of its host, specimens are sometimes found on the outer surface. Probably this only occurs under abnormal conditions.

Like Histriobdella, as stated by Shearer (**9**, p. 297), Stratiodrilus readily deserts its host when conditions are in the least abnormal. When the crayfish is kept for a little time in a vessel with a limited amount of water, Stratiodrili will frequently be found crawling on the bottom of the

vessel, and it often happens that under such circumstances none are to be found in the branchial chambers.

BODY-CAVITY.

Surrounding the enteric canal, between the wall of the latter and the wall of the body, is a space of considerable size, which occupies the position of the cœlomic cavity of the Annulata. This is referred to by Fœttinger (**3**, p. 490) as the " cavité générale du corps," and is described by him as a cavity between the musculo-cutaneous tube and the digestive— bounded by somatopleure and splanchnopleure, which are stated to be thin cellular membranes with flattened nuclei. A corresponding cavity in the head is said to be completely cut off from the body cavity of the trunk by a septum composed of large cells. The somatopleure of the trunk is described as clothing the dorsal and ventral muscles and the nerve-cord.

Shearer (**9**) terms the cavity in question blastocœlic cavity on the assumption that it corresponds developmentally with the similarly situated cavity in Dinophilus, and that the latter is of blastocœlic derivation. He describes prolongations into the head and into the posterior limbs. He doubts the correctness of the view that it is lined by a cœlomic epithelium.

The gut surface, he admits, is lined by a delicate " cuticle " with small flat nuclei at rare intervals, and he maintains that it is difficult to say if this is a membrane or a " mere secretion from the blastocœlic ends of the cells of the gut-wall." The somatopleural side of the cavity, he asserts definitely, is not lined by any such membrane : the longitudinal muscles, as in Stratiodrilus, are surrounded by a delicate cuticle similar to that which lines the outside of the gut, but no nuclei are to be seen in it. He maintains that neither the splanchnic nor the somatic layer is of the character of a true peritoneal or cœlomic epithelium.

I have not yet been able to work out definitely the

development of the corresponding cavity in Stratiodrilus, but what evidence I have before me seems to point rather to a schizocœlic origin than a blastocœlic derivation. However this may be, the layer which forms its splanchnic boundary is assuredly of the nature of a nucleated membrane. The presence of the nuclei, though there are wide intervals between them, definitely proves untenable the view that this layer can be looked upon as a cuticle or as a secretion from the cells of the enteric epithelium. The somatic layer also is in a part of its extent quite unmistakeably a membrane of similar character. Forming the ventral wall or floor of the body-cavity is a thin membrane of material similar to that which forms the splanchnic layer, and with similar flattened nuclei. On the dorsal side this membrane does not exist, and the nuclei, if represented at all, are represented by nuclei which lie embedded in protoplasm on the inner surfaces of the muscular fibres.

To avoid the misconception that in this substance lying between the epidermis and the enteric epithelium we have to do with a definite epithelium, I propose to use the term cœlenchyme introduced by Salensky, and applied later by de Beauchamp (1) to a very similar tissue occurring in Dinophilus. The cœlenchyme is to be found in what is probably its most significant, because its most primitive, condition in the tail region (fig. 15 c. and figs. 2–6 c. c.). Here, as in the trunk, it forms a definite splanchnic layer surrounding the enteric epithelium of the intestine. But there is no distinct somatic layer. Instead there is on either side an irregular strand running forwards from the corresponding rectal gland, and connected with its fellow of the opposite side by a transverse strand, which is intimately united with the dorsal surface of the nerve-cord. In each of the lateral strands runs the caudal part of the corresponding third nephridium. From each lateral strand branches are given off which become continuous with the longitudinal muscular fibres, both dorsal and ventral—a nucleus occurring in each of these branches close to its termination in the muscular fibre. On

each side the branches to the dorsal fibres are given off from
an almost vertical lamina, through the ventral part of which
the nephridium runs. Enclosed between these two laminæ
on the ventral side of the intestine is a longitudinal channel,
which forms the chief part of the body-cavity in this region.
Further forwards, towards the anterior limit of the caudal
region, the laminæ of the cœlenchyme become exceedingly
thin, and the branches passing to the dorsal series of
muscular fibres appear as offsets from the splanchnic layer,
those to the ventral fibres being, as before, given off from
the main longitudinal strand in which the nephridium runs.

At the point where the tail passes into the trunk the
relations of the cœlenchyme and the muscular fibres become
somewhat complicated owing to the development of an
oblique septum, partly muscular, dividing the body-cavity in
this position; and in the reproductive segments in front of
this a similar degree of complication is brought about in the
female by the presence of the large ripe ovum, and in the male
by that of the penis, accessory glands and vesiculæ seminales.

In the male in the posterior part of the genital region the
most considerable part of the cœlenchyme takes the form of
a pair of wing-like, nearly horizontal, lateral plates projecting
out from the splanchnic layer covering the intestine, and
giving off branches to the dorsal muscular fibres. The
cœlenchyme does not here form a continuous somatic layer
on either surface. Further forwards there are formed a pair
of vertical partitions, partly muscular, separating off a
median chamber containing the intestine, the nerve-cord,
the penis and a median part of the testis, from two lateral
chambers enclosing the lateral portions of the testes, the
vesiculæ seminales and the prostate glands. In this part the
cœlenchyme only gives rise locally and imperfectly to a
somatic layer. The splanchnic layer is reflected over the
testes, etc.

In the female the posterior part of the genital region is so
distended by the enormous ripe ovum (fig. 10) that the other
parts become reduced and compressed, and the cœlenchyme

is condensed to form definite somatic and splanchnic layers, the former closely applied to the muscular layer of the body-wall, the latter giving rise to an investment for the ovary in addition to the usual layer investing the narrowed intestine, here sunk in the dorsal body-wall.

Further forwards (fig. 9) a pair of vertical longitudinal septa are formed very much as in the male, the median chamber enclosed by them being occupied by the intestine, the nerve-cord, and the ends of the loops of the third pair of nephridia, the lateral chambers lodging the lateral parts of the ovaries. The median chamber is crossed by strands of cœlenchyme, the most constant of which is one that forms a horizontal partition bounding dorsally a channel (partly divided by a median vertical partition) running immediately above the nerve-cord. The somatic cœlenchyme here forms a definite membrane on the ventral side; a splanchnic layer invests the ovaries.

In the anterior trunk region (fig. 7) the vertical septa become replaced by oblique septa (of cœlenchyme with occasional muscular fibres).

These extend from the middle of the dorsal body-wall, and run outwards and downwards, holding the stomach between them as they diverge, and are inserted into the lateral parts of the ventral surface. There is a continuous somatic layer on the ventral side closely related to the ventral longitudinal muscles, circumscribing them into two distinct bundles.

The body-cavity extends through the neck-segment into the head (fig. 13 and figs. 1 to 6). Septa of cœlenchyme with muscular fibres which run nearly transversely separate the cœlom of the neck-segment from the cœlom of the head in front (S. 5) and from that of the first trunk-segment behind (S. 1). Whether these form complete partitions is doubtful. The fact, recorded in my previous paper, that spermatozoa occur sometimes in the interior of the head-cœlom is not conclusive as to the existence of apertures in the septa, since the sperms might have been directly injected into the head. The neck-cœlom, certainly, under certain circumstances, as

described below in the account of the cervical glands, behaves like a closed cavity. In the head the cœlom is represented by an extensive cavity on the ventral side, with lateral extensions round the jaws and their muscles, opening dorsally into a considerable median cavity situated beneath the brain. These head-cavities are quite clear except for some muscular fibres. They have a splanchnic layer of cœlenchyme like that covering the intestine, forming a capsule enclosing the jaws and their muscles, and the existence of a parietal layer is indicated by the presence of very sparsely distributed nuclei.

Briefly stated, the arrangement of the cœlenchyme and its relations to the cœlom in Stratiodrilus are as follows: The cœlom, which is probably a schizocœle, is not lined in any part by an epithelium; but the cœlenchyme, a nucleated substance of undifferentiated, finely fibrillated material, with no trace of division into cells, partly takes the place of such a membrane. It forms a thin splanchnic layer investing the whole of the digestive canal and the ovary and testis. Its somatic portion, which assumes the character of a continuous layer on the ventral side only, is intimately connected with the longitudinal muscular fibres of the body-wall, of which it constitutes the formative (myoblastic) material.

As I pointed out in my previous paper, this condition, in which the same elements play the part of myocytes and of somatic cœlomic epithelium, is essentially not dissimilar to the condition in the larva of Polygordius (Fraipont, 4), in which a single layer of cells gives origin both to the longitudinal muscular fibres and the somatic layer of the cœlomic epithelium. To judge from certain of Eisig's figures (2), the same holds good of the Capitellidæ.

Pierantoni (8) describes a complete peritoneal layer in Protodrilus, but does not enter into an account of its relationships to the muscular layers. In his text-fig. 1, p. 33, he shows a cell with a flattened nucleus lying within and distinct from the protoplasmic parts of the longitudinal muscular fibres, and refers to it as representing the peri-

toneum. From this we might infer that a distinct separate layer of such cells lines the cœlom, but the figures of sections given in the plates—e. g. the figures on Pl. 7—do not show this, and represent only a single set of nuclei.

But there are also adult forms among the Chætopoda in which the condition is remarkably like that occurring in Stratiodrilus. In an Enchytræid not yet determined, which is very common in Sydney in moist garden soil, there is, as represented in fig. 29, in many parts only one layer, not composed of cells, but of a nucleated material which is not unlike the cœlenchyme of Stratiodrilus, doing duty both as the myocytes of the longitudinal muscular fibres and also as the somatic cœlomic layer. But in some parts this material is divided into two layers—an outer, surrounding the inner edges of the muscular fibres, and containing nuclei situated in close contact with the latter, and an inner, provided with nuclei flattened tangentially, situated at irregular intervals. The substance of these two layers is in great measure in continuity, the filaments of the protoplasm being traceable from one to the other, and in some parts the coalescence appears to be complete, so that only one layer is recognisable.

In this Encytræid, then, the relations of the somatic peritoneal layer are either more primitive than in the Oligochæta in general, or have become secondarily modified, and are closely comparable to what occurs in Protodrilus, approximating to a certain extent towards the condition prevailing in the Histriobdellidæ (fig. 28). It seems to me highly probable that in this respect, as in the relations of the nervous system to the epidermis,[1] the more primitive, or simplified, condition may prove to be by no means exceptional among the smaller and simpler Chætopoda.

Jaws and Digestive System.

The jaw-apparatus of the Histriobdellidæ is an extremely complex structure. Altogether some thirty distinct

[1] See E. S. Goodrich, " On the Structure and Affinities of Saccocirrus," ' Quart. Journ. Micr. Sci.,' N.S., vol. 44, p. 422.

chitinous pieces with elaborate articulations enter into its composition.   It is thus a much more complex structure than the mastax of certain Rotifers to which I have compared it. Yet the resemblances to the "malleate" form of mastax are very striking, and are worthy of notice in connection with the question of the phylogeny of the Histriobdellidæ.

Compared with the typical malleate mastax, as we find it exemplified in Brachionus, the jaw-apparatus of Stratio-drilus is found to differ in the following chief points:

(1) The fulcrum is produced into a long slender rod.

(2) Each ramus is represented by four more or less parallel jointed rods or ramules, each of which bears terminally one of the four complex teeth which represent the uncus.

(3) The uncus is thus essentially related, not to the manubrium, but to the ramus.

(4) The manubrium is probably represented by a very slender rod which articulates with the ramus towards its distal end and extends outwards.

(5) The lower jaws of the Histriobdellidæ are apparently not represented at all in the mastax of the Rotifer, unless we are to look on them as corresponding to greatly developed posterior parts of the manubria.

(6) The entire apparatus, it is important to note, is situated, not in the interior of the enteric canal as in the Rotifer, but in a blind pouch (pharynx) which lies on the ventral side of the œsophagus and opens on the exterior through the mouth. In Paraseison alone among the Rotifera does the mastax occupy a corresponding position.

I am able to supplement my earlier account of the jaws of Stratiodrilus by certain additional particulars regarding the rami of the upper jaw and to give more detailed figures of them (figs. 26, 27). Each ramus consists of four sets (ramules) of movably articulated chitinous pieces, each ramule ending in a "tooth." Of these four teeth only the outer two have the curry-comb-like character to which I previously directed attention (6, p. 307). The other two are

beset on the inner aspect with a row of sharp denticles, four in number on the outer tooth, eight on the inner. All four ramules of each ramus are connected together by articulations, so that they act together in the movements of protrusion and biting.

The jaws of S. novæ-hollandiæ differ from those of S. tasmanicus in certain respects. In most cases, as already mentioned, the entire apparatus is considerably longer in the former than in the latter. The individual chitinous parts also present definite and constant differences. The most marked of these concerns the broad plate which terminates each of the lower jaws in front. The anterior margin of this plate, provided with about six irregular denticulations in S. tasmanicus (fig. 16), is in S. novæ-hollandiæ (fig. 17) marked with a deep, rounded incision near its inner margin, followed by a comparatively large tooth, which is separated by a shallow notch from a second, much smaller tooth.

The muscles of the jaws have been described in Histriobdella by Shearer and in Stratiodrilus by myself, and the agreement between the two genera is fairly close. The chief muscles in Stratiodrilus are in three sets. One of these represents what Shearer calls in Histriobdella the " bulblike muscular organ of the jaws." In Stratiodrilus I described it as " a pair of large bundles of non-striated fibres, each of which is wrapped round the ventral side of the corresponding lower jaw, the fibres running forwards parallel with the latter throughout their (its) length. These two muscles are in close apposition with one another along the mid-ventral line, separated, however, by a thin septum of nucleated material continuous with the lining of the head cœlom, of which it appears to be a thickening. They are continuous with the retractor fibres behind. The ventral edge of each is unfolded, and becomes continuous with the ventral edge of the corresponding muscle of the second pair "—to which account has to be added the statement that these muscles are continued from the lower jaws almost vertically upwards to

the dorsal wall of the head, embracing between them the œsophagus and the dorsal cephalic gland (fig. 13). In the living animal, when the fibres of these muscles contract, there are synchronous movements of the margins of the mouth.

The second pair are the striated muscles (figs. 2 and 13, *s. m.*), the fibres of which arise from the shaft of the lower jaw towards its posterior end and run straight forwards to be inserted into the corresponding bridle—a curved chitinous piece that slides backwards and forwards along the shaft and probably serves as the medium through which the contractions of the striated tissue are transmitted to the rami of the upper jaw (see **6**, p. 309).

A third set of muscular fibres arise from the fulcrum of the upper jaw, and run forwards parallel with it to be inserted into the rami. These apparently bring about restricted biting movements which sometimes occur when the entire jaw apparatus is not fully exserted, the chief biting movements, with the jaws in a state of complete protrusion being probably due to the contractions of the striated muscles inserted into the bridles.

A number of muscular fibres arising from the wall of the head in the posterior part of its extent about the bases of the anterior limbs pass through the cœlomic space that underlies the brain, partly in relation to its dorsal, partly to its ventral, surface, and are inserted into the rami. In addition, retractor and protractor strands pass to the apparatus from the walls of the head.

The digestive system is much the same in essentials in Histriobdella and in Stratriodrilus. The whole system, apart from the jaws and certain glands to be referred to presently, consists of a simple tube (figs. 1 to 6) running straight through from mouth to anus. The first part, the œsophagus, situated in the head, is an extremely narrow tube. The second part, stomach, running through the anterior trunk region, is much wider. Then follows a very narrow anterior part of the intestine running through the reproductive seg-

ments, and finally a wider intestine running through the tail
region and terminating in the anus at its posterior end on
the dorsal side. The wall of the canal consists throughout
of a single layer of comparatively large cells ciliated internally
and a thin investment of the splanchnic cœlenchyme, already
referred to. Here and there is a cell which responds much
more intensely than the rest to staining agents, and which
may perhaps be differentiated as a secretory cell.

Connected with the œsophagus are a pair of glands (figs. 2–5,
gl. c.), not hitherto noticed which from their position may be
termed the c e r v i c a l g l a n d s. These are situated in the neck-
segment at the sides of the œsophagus in the interval between
the jaws in front and the intestine behind. In the living
animal they appear as somewhat conspicuous objects owing
to their greenish colour, but a very slight pressure causes
them to become broken up, when the green matter becomes
diffused through the space (cœlom of neck segment) bounded
by transverse septa in front and behind. Each gland is
triangular in outline when looked at from above or below,
one angle being internal and the other two external. The
internal angle is in close apposition with the narrowest part
of the œsophagus, where it passes into the intestine. In
section the gland appears very finely fibrillated, not showing
any trace of division into cells, with only a small number of
nuclei placed at wide intervals. In one series of sections the
gland appears to be unicellular with a single large nucleus,
larger than any others that occur in the head-region, with a
definite spherical nucleolus; any other nuclei towards the
periphery belonging to other elements. In other series this
point is not so distinct. The two glands are closely applied
together below the œsophagus, separated by a very fine
median vertical fissure. Closely applied to their ventral
surfaces are the transverse parts of the first or cephalic pair
of nephridia, and between this surface and the ventral body-
wall is a distinct space—the cœlom of the neck-segment
(fig. 1, *ce. c.*).

Comparison of series shows that these glands are essentially

unicellular, but may become plurinuclear by division of the original single large nucleus. The bodies above described are not referred to either by Fœttinger or by Shearer, though they are distinctly shown in the figures of longitudinal sections by the former. They are entirely distinct from the salivary glands of the latter author, which also occur in Stratiodrilus, and lie further forwards in close relation to the jaws and their muscles. Their physiological connections may be with the nephridial and not the digestive system.

Another conspicuous structure in this region, not represented or not recognised in Histriobdella, lies on the dorsal side of the head, a little further forward. This is a median organ (figs. 4 to 6 and 13, *gl. d.*), deeply divided behind into right and left lobes, which lie at the sides of the œsophagus, and are thus dorsal to the jaws. The unpaired part very closely invests the dorsal surface of the œsophagus. In front it becomes divided into three distinct lobes, giving off dorsally narrow processes, which end in the epidermal layer.

The nature of this organ—the dorsal gland—is doubtful. Its substance stains deeply with hæmatoxylin, when it presents a vacuolated appearance, suggesting that of a gland for secreting mucus. It is not divided distinctly into cells, and contains only a few nuclei, which are very similar to those of the cells of the glands belonging to the anterior limbs.

Yet another pair of glands which have not been noticed hitherto may be referred to here. These are a pair of spherical masses of cells (figs. 4 to 6, *r. gl.*), each situated at the side of the anus in close relation to the corresponding posterior limb-gland. The cells have a generally radial arrangement, but I have found no lumen, and have not been able to trace any duct, and there is no definite evidence that these glands, which may be termed on account of their position the rectal glands, have any outlet.
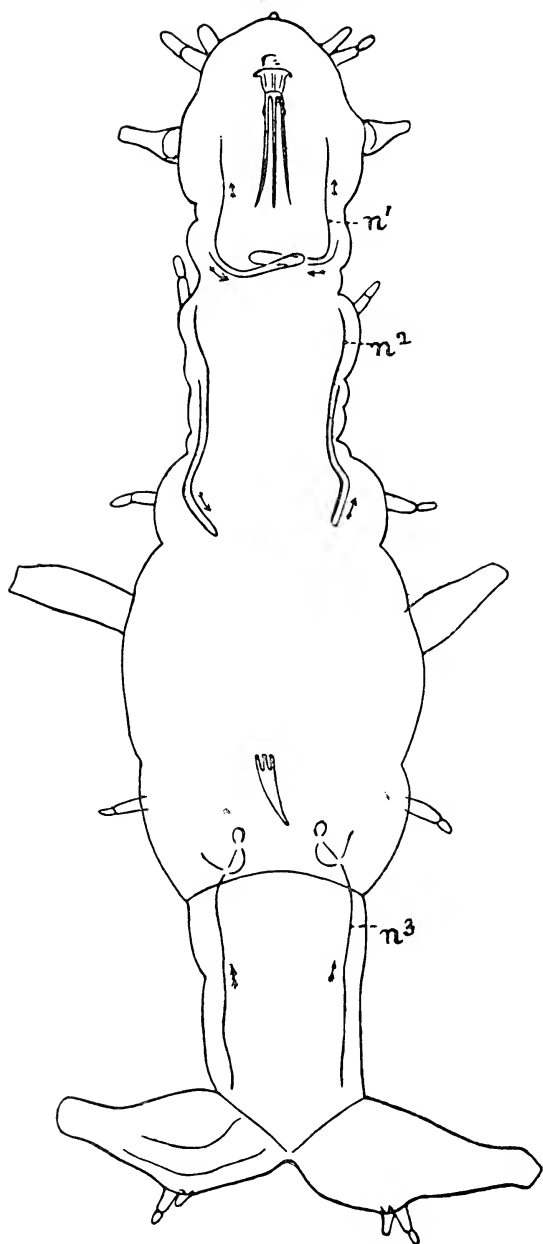
Outline of male Stratiodrilus novæ-hollandiæ to show the arrangement of the nephridia, n^1, n^2, and n^3. The arrows indicate the direction of the ciliary movement.

The Nephridia.

There are three pairs of nephridia (Text-fig. 1) in both
male and female. The nephridia of the first pair (head
nephridia) are alike in both sexes. Each begins in the
lateral part of the neck segment, not far from the lateral
border. In some series of sections each tube seems to have
its origin in a thin-walled vesicle. From its point of origin
the nephridial tube runs inwards with a curve, the con-
vexity of which is directed backwards, and crosses the
middle line, running ventral to the œsophagus and some
little distance behind the posterior extremities of the jaws.
After crossing the middle line the nephridium extends for
a little distance further in the same direction, and then bends
sharply round on itself, forming a complete loop, and running
back almost parallel with and somewhat in front of its
first-mentioned inwardly trending limb, till it reaches a point
not far from the origin of the latter, when it again changes
its course to run straight forward into the head. In the
head the nephridium is traceable by means of its contained
cilia to a point nearly opposite the middle of the base of
the anterior limb, where the ciliary movement is always
found to cease in a position far removed from either surface
of the head. In sections the tube is traceable to a point
just in front of the cephalic limb, where it comes close to
the ventral surface and terminates there. It is at this
point doubtless that the external aperture is situated.
But apparently in this terminal non-ciliated part of the
nephridium the lumen becomes divided up into a number
of extremely minute channels, of which the openings on
the surface would not be traceable. The wall of this
nephridium is very delicate throughout, and it is in few series
of sections that the arrangement can be followed. Where
the loops overlap, on the ventral aspect of the neck-segment,
the nephridia have the appearance of narrow channels lined
by cilia running through a mass of fibrillated material, in
which a nucleus is embedded here and there.

The second pair of nephridia (n^2.) begin in both sexes close to the bases of the first pair of cirri, but dorsal to the latter; in some cases each appears to originate in a small contractile vesicle. From this point each runs backwards through the rest of the second segment and through the whole of the third. In the male it bends inwards at a point nearly opposite the base of the second cirrus (third segment), and, after running inwards and backwards until it approaches very near its fellow of the opposite side, it bends sharply round on itself a little distance in front of the clasper. From the bend it runs forwards in close relation to its posteriorly running limb and on its outer side. The external opening of this nephridium has not been observed in the male. The cilia of the anteriorly running limb cease a little distance in front of half the interval between the first and second cirri, and the last cilia are a considerable distance from the surface, so that the terminal part here, as in the head nephridia, would appear to be non-ciliated or to possess cilia that are not usually in movement. In the female the second nephridium is continued further back than in the male, the loop or bend (fig. 7) occurring nearly opposite the anterior paired part of the ovary. The anteriorly running limb of the loop runs throughout on the inner side of the posteriorly running limb, quite close to the latter as seen in transverse sections, and opens on the exterior on the ventral surface near to, and a little in front of, the second cirrus. In one transverse series the part near the opening is loaded with a quantity of amorphous, opaque matter. The terminal part is a coiled non-ciliated tube.

The third nephridia (n^3.) in both sexes begin at the posterior end of the tail region. In line with each, behind the point where the lumen begins, is a row of four or five large nuclei, situated at the side of the rectum. It runs forwards on the ventral side between the nerve-cord and the intestine till it enters the trunk. In this position the vessel is quite distinct in thin sections (fig. 15). In the female the trunk part of the nephridium runs forwards on the dorsal side of the ovary to a point near the anterior extremity of the latter,

where it bends round and runs inwards and forwards below
the intestine to form a loop (figs. 8 and 9), which approaches
near to or even meets that of the opposite side in a space
between the intestine and nerve-cord, bounded laterally by a
pair of almost vertical septa and separated from a space
immediately overlying the nerve-cord by a horizontal septum.
The returning limb of the loop is dorsal and, further back,
internal to the other ; it runs back, and is traceable as far as
a point nearly opposite the third cirrus and about half way
between the lateral border and the middle line.   Its external
aperture on the ventral surface must be a little behind the
third cirrus, but the absence of cilia in the terminal part makes
it difficult to fix this point definitely, and this part of the
nephridium, owing apparently to the crowding produced by
the presence of the reproductive apparatus, is extremely hard
to follow even in the best series of sections.

In the male the trunk portion of the third nephridium forms
a loop which does not extend as far forward as the penis and
seminal vesicle.   The returning limb of the loop, which may
be twisted on itself, passes towards the ventral side and the
cilia terminate a little distance behind the base of the third
cirrus.

The above account of the nephridial system of S. novæ-
hollandiæ differs in certain important points from that which
I have previously described as the arrangement in S. tas-
manicus—particularly as regards the first and second pairs.
Whether these discrepancies are due to actual differences in
the two species, or, as seems more probable, to misinterpreta-
tion of the appearances previously observed, can only be
determined by the re-examination of the Tasmanian species
in the living condition.

The differences between the nephridial system of Histriob-
della as described by Foettinger (3) and more recently and
more fully by Shearer (9), and that of Stratiodrilus are of
a very marked character.   In the former the system does not
extend either into the head or into the tail, and the three (♀)
or four (♂) pairs of tubes of which it is composed are simple,

approximately straight, and run in all cases from before backwards to open on the ventral surface.

In the occurrence of a pair of nephridia in the head region in the adult condition, Stratiodrilus differs not only from Histriobdella, but from every other group with which it might be supposed to have affinities. The extension of each of these head-nephridia in a loop across the middle line is also a very special feature. Perhaps this may be explained by the very intimate relationship that appears to exist between these loops and the structures which I have called the cervical glands, and which, as I have already suggested, may belong to the nephridial system.

Both Foettinger and Shearer describe the nephridia as intra-cellular tubes, and the former definitely states (p. 471) that the section has the appearance of a rounded nucleated cell presenting an aperture in its cytoplasm. But such a description does not convey an accurate impression of the actual nature of these organs—at all events as they occur in Stratiodrilus. The tissue through which they run is not cellular in the strict sense of the term. It consists of a differentiated part of the nucleated cœlenchymatous tissue not divided into cells, the outer portion of which has the function of the myoblastic tissue for the muscular fibres of the body, while the inner forms a thin layer investing the gut and having the relations, though not the structure, of a splanchnic epithelium. Throughout a whole series of sections of the caudal region there are no nuclei that have any special and intimate relationships with the walls of the nephridial tubes. Further forwards in the same pair of nephridia, where the walls have assumed greater definiteness, and where, at long intervals, nuclei appear in this wall, there is still no question of cells, but simply of a greater condensation of the tissue around the lumen of the nephridia.

Foettinger describes the vibratile structures in the interior of the nephridia as cilia. But, as Shearer has pointed out, they have much more the appearance of elongated flagella. The relationships of these are very difficult of determination,

and, in common with Shearer, I find myself quite unable to
come to a decision as to their connections.

NERVOUS SYSTEM.

The remarkable development of the nervous system in the
Histriobdellidæ, and especially in Stratiodrilus, dis-
tinguishes them widely from any lower group with which we
can compare them, and, if not constituting strong evidence of
annulate affinities, is a very remarkable instance of convergent
development. In Histriobdella this system was described
with great thoroughness and completeness by Foettinger, and
additional details were given by Shearer, and a careful com-
parison with the nervous system of Dinophilus gyro-
ciliatus as described by Nelson (7).

The nervous system of Stratiodrilus novæ-hollandiæ
closely resembles that of S. tasmanicus. The cerebral
ganglion (figs. 2 to 6, b.) contains a massive neuropile situated
in the head towards the dorsal surface, somewhat in front of
the middle of the region and well behind the mouth. In
sagittal section this appears ellipsoidal in outline, with a
slight dorsal depression indicating a division into anterior and
posterior lobes. In horizontal section it is seen to be some-
what more elongated transversely than antero-posteriorly, and
to be divided by a wide posterior notch into two lateral lobes.
Applied to the neuropile dorsally and laterally are a great
number of nerve-cells. These extend over the greater part
of the head, and are continued backwards for some distance
along the œsophageal connectives. This layer of nerve-cells
is distinct from the epidermis, which is continued as a definite
layer, with an occasional characteristic nucleus, between it and
the surface. At the same time it lies distinctly outside the
neuropile, and thus the appearance which the brain as a
whole presents in sections is very characteristic, and differs
in a very marked manner from the appearance presented by
corresponding sections in the case of a Chætopod. Probably
in this, as in so many other points, there is a closer connection

with Dinophilus than with any other annulate animal (see Nelson, 7, pl. 13, fig. 20).

Opposite the base of each of the tentacles is a group of nerve-cells which give off processes outwards into the tentacle and inwards into the neuropile.

There is a distinct rudimentary visceral nervous system (fig. 13, *r. n.*) similar to that described by Foettinger as occurring in Histriobdella. It consists of a pair of strands of nerve-cells given off from the œsophageal connectives close to their origin from the brain and passing back to the jaws and their muscles.

The œsophageal connectives pass downwards and backwards at the sides of the œsophagus and jaws to meet towards the ventral side of the neck-segment in the first ganglion of the ventral chain. Each gives off, as above stated, close to its origin from the brain a visceral nerve, which, accompanied by a number of nerve-cells, runs backwards among the muscles of the jaws.

The first ganglion of the ventral chain (figs. 1–6, *n. c.*), situated in the neck-segment, is a very small one and is scarcely separated from the next. The ganglion of the second segment is of large size, and extends throughout the greater part of the length of the segment. Throughout the greater part of the length of the second segment the nerve-cord is double, being divided by a median vertical fissure, which is continued into the connective between the second ganglion and the third. Laterally the second ganglion gives off a pair of nerves, passing to the small ganglia at the bases of the cirri of the first pair. The third ganglion is also a large one, and is not divided by any median fissure : it gives off the nerves to the second cirri. The connectives between the third ganglion and the fourth, which are shorter than those in front, are separated by a well-marked median fissure. The fourth ganglion in the male corresponds to the claspers, to which it gives off a large offset. In the female the corresponding nerves end in a pair of lateral ganglia. The succeeding pair of connectives are separated from one another

by a narrow, but distinct, fissure. In the female the nerve-
cord is much compressed in the fifth segment, in which the
single mature ovum is formed. In the male, in a correspond-
ing position in the fifth (or second reproductive) segment, the
cord bends away from the ventral surface and passes on the
dorsal side of the vasa deferentia and penis to resume its
ventral position behind them. The fifth ganglion, the last of
the trunk, gives off nerves to the cirri of the third pair. The
connectives following this are very short and are in close
apposition: they correspond in position to the partition
between the trunk and the tail. In the tail region are a
series of rather small ganglia which are in very close apposi-
tion, the intervening connectives being very short and in a con-
tracted specimen scarcely distinguishable. These five small
ganglia correspond to the five imperfect segments into which
this region is divided by slight external transverse constric-
tions. Each of these ganglia has connected with it laterally
a pair of small ganglia corresponding serially with the lateral
ganglia in the segments of the trunk. Posteriorly the nerve-
cord divides into two branches, one passing into each
posterior limb, each giving off a branch to the corresponding
limb-cirrus.

REPRODUCTIVE SYSTEM OF FEMALE.[1]

S. novæ-hollandiæ has a pair of extensive vitelline
glands (figs. 7–9, *vit.*) corresponding to those of S. tasma-
nicus (**6**, p. 322, fig. 11, *vit.*) These are a pair of irregularly
shaped masses, of deeply staining protoplasmic material, partly
divided into several elongated narrow lobes, without cell-
boundaries, but with nuclei at irregular intervals. They are
dorsally situated in close relation to the dorsal bundles of
longitudinal muscular fibres, and they extend from a point
just opposite the second cirrus throughout the greater part
of the length of the genital segments, each entering the
corresponding ovary and terminating in a leash of slender

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[1] I have nothing to add to the account previously given by me of the
male reproductive apparatus.

processes (ducts) in the neighbourhood of the posterior part of it. They do not contain any yolk-granules as such, and the latter must be formed within the growing ovum itself, the secretion providing the requisite material. That they do perform this function I conclude from their situation, and from the fact that they are the only organs of sufficient bulk to be capable of producing with rapidity the relatively considerable mass of substance which the ovum has to receive before it reaches maturity.

In mature females there is nearly always a single ovum (figs. 1–6, and 10, 11, *ov.* [1]), which is very much larger than the second in size, and which causes the distension of the region of the body in which it lies and the compression of the other organs—intestine, nerve-cord, and muscular system. The cavity in which it lies, a part of the cœlom, is situated between the cirri of the third pair. A pair of forward extensions of this cavity enclose the rest of the ovary in the shape of right and left lobes. The entire ovary is enclosed in a thin layer of splanchnic cœlenchyme, which is produced inwards in such a way as to form an investment for each of the fully formed ova. Each ovarian lobe contains a single row of ova, diminishing in size from behind forwards. This row bends inwards at the apex of the lobe, and turns back on the inner side to become merged in a nucleated layer which is not separable from the cœlenchymic investment, and in which occasional mitotic figures are to be detected.

The principal ovum is loaded with large spherical yolk-granules, and in some cases the ovum next in size contains more or fewer of these bodies; in the rest yolk-granules are absent. In sections which have been well stained with hæmatoxylin the outermost layer of the largest ovum, and usually that of the next in size, is darkly coloured by the dye, and appears as an investment of a vacuolated character sending numerous short processes inwards. This is obviously not a special investment, but a superficial protoplasmic layer having an affinity for a nuclear stain that in all other parts affects in the same degree only the nuclei.

The efferent part of the female apparatus of Stratio-
drilus differs considerably from that of Histriobdella as
described by Foettinger, whose account is endorsed by
Shearer. It was incorrectly described in my former paper,
since I mistook the returning limbs of the third pair of
nephridia for the oviducts. There are two female apertures
situated ventro-laterally a little distance behind the third
pair of cirri (fig. 14, ♀). Each leads into a short passage,
which in some cases shows a rounded dilatation. Around the
aperture, adherent to the integument, is an irregular layer
of amorphous clear-looking matter (Sh.), which may partly
block the passage itself. I am inclined to agree with
Foettinger that this must be shell-forming material, and that
it is produced by the specially developed epidermal layer
around and within the aperture; it does not possess the
resistent character of the egg-shell itself; but that would, as
in other cases, only be acquired on contact with the ovum—
probably as the result of some influence exerted by the outer
layer of the protoplasm. The passage or oviduct opens
internally into a compartment of the body-cavity bounded
behind by the septum separating the trunk-cœlom from the
tail-cœlom, and in front by a second partition of cœlenchyme
bounding behind the cavity in which the ripe ovum is lodged.
The aperture does not take the form of a funnel as in
Histriobdella; but, just in front of it on its dorsal side
is a prominent cushion-like body (cu.), the surface of which
is beset with stiff cilia vibrating sluggishly in the living
animal. In the cushion and around the aperture are nume-
rous nuclei and many muscular fibres.

The partition (s.²), separating the cavity in which the
ripe ovum (with the rest of the ovary) lies from that out of
which the oviducts lead, is thickened towards the middle, and
produced into a pair of more or less prominent processes
projecting backwards and outwards into the cavity behind.
These thickenings, which vary greatly in different specimens,
contain a number of nuclei, together sometimes with rounded
bodies staining deeply with eosin. The proximity of these

to the oviducts and their cœlomic apertures suggests that they may have some function in connection with the discharge of the ovum.

In living specimens active movements of a remarkable character were repeatedly observed to be taking place in the largest ova. The appearance presented is that of a narrow process being actively pushed inwards from the adjacent tissues towards the centre of the ovum ; but since there is no apparent mechanism present by which such an effect could be produced, the movements must be the result of contractions of the substance of the ovum itself—probably of the specialised superficial layer already referred to. The effect of such movements is to bring the centrally placed nucleus within easy reach of the periphery. Two ends might presumably be met by this peculiar change; the polar bodies might be separated off without the nucleus being forced to travel from its usual central position through a dense mass of yolk-granules to the periphery, or the spermatozoon might be at once received into the neighbourhood of the nucleus of the ovum without having to perform a like journey.

Only in one case have I obtained sections of a specimen fixed while such movements were going on. In this case it was the second largest ovum—here thickly beset with yolk-granules, in which, as observed also in a living specimen, the phenomenon occurred. The ingrowth in this case reaches from the outside nearly to the centre of the ovum, and appears as a narrow fissure filled with cœlenchyme. The nucleus of the ovum, situated near the bottom of this fissure, has become modified ; it has become distended, the nuclear membrane has almost disappeared though still traceable, and the chromatin has become broken up into numerous small granules. Whether the circumstance that in this specimen numerous spermatozoa are to be detected fixed in the act of wandering through the cœlenchyme in the immediate neighbourhood of the fissure in the ovum has anything to do with the peculiar change in the latter must remain undetermined. For the investigation of this problem and the following out of the history of the

maturation and fertilisation, a much more abundant material is required than is at present available.

Masses of sperms are almost always to be found on each side in front of the third cirri and genital apertures. The appearances which these present vary very greatly. In one of the series of sections of the Tasmanian species previously described by me, the main mass of sperms is contained in what has the appearance of a case corresponding in form to a cast of the interior of the penis (6, pl. 15, fig. 12). This I supposed to be a spermatophore, and a re-examination of the sections strengthens the conclusion. In some series of sections of S. novæ-hollandiæ similar appearances are to be observed, though here the case of the spermatophore, if such it be, is rounded in form. In other series no trace of such a case appears. In one instance a stream of sperms is traceable backwards to the dorsal surface, where it is continued through the integument. It would thus appear that in the process of hypodermic impregnation the case of the spermatophore may or may not be passed into the interior of the impregnated female.

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EXPLANATION OF PLATES 11 to 14,[1]

Illustrating Professor **W. A. Haswell's** "Notes on the Histriobdellidæ."

LIST OF REFERENCE LETTERS.

b. Brain. *bd.* Bridle. *c.* Cœlenchyme. *c.c.* Caudal cœlenchyme.
ce.c. Cervical cœlom. *ci.* Cirri. *cu.* Ciliated cushions. *f.* Fulcrum.
gl.c. Cervical gland. *gl.d.* Dorsal gland. *h.c.* Head-cœlom. *i.* Intestine. *j.* Jaws. *l.gl.*[1]. Gland of anterior limb. *l.gl.*[2]. Gland of posterior
limb. *l.j.* Lower jaw. *m.d.* Dorsal longitudinal muscles. *m.v.*
Ventral longitudinal muscles. *n.*[1], *n.*[2], *n.*[3]. First, second and third pairs
of nephridia. *n.c.* Nerve-cord. *o.* Ovary. *œ.* Œsophagus. *ov.*[1]
Largest ovum. *ov.*[2]. Second largest ovum. *r.*[1], *r.*[2], *r.*[3], *r.*[4]. Ramules of
upper jaw. *r.gl.* Rectal gland. *s.*[1]. Septum between neck-segment and
trunk. *s.*[2]. Septum behind large ovum. *s.*[3]. Septum between trunk and
tail. *s.*[4]. Partial segment in front of largest ovum. *s.*[5]. Septum between
neck-segment and head. *sh.* Shell-gland secretion. *sh.gld.* Shell-gland.
st. Stomach. *s.m.* Striated muscle (of jaws). *sp.* Spermatozoa. *sph.*
Sphincter between œsophagus and stomach. *spp.* Spermatophore.
u.j. Upper jaw. *v.m.* Vertical or dorso-ventral muscular fibres. *v.n.*
" Visceral " nervous system. *vit.* Vitelline glands. ♀. Female genital
aperture.

PLATE 11.

Figs. 1–5.—Five successive longitudinal vertical sections of a mature
female Stratiodrilus novæ-hollandiæ. × 200. In the sections
represented in figs. 1 and 4 the lower jaw (*l.j.*) has become detached from
its natural position.

PLATE 12.

Fig. 6.—A sixth section of the same series as those represented in
Plate 11. × 200.

[1] All the figures were re-drawn for me by Mr. F. W. Atkins.

Figs. 7–12.—Transverse sections of a mature female specimen of S. novæ-hollandiæ. × 600. The cilia of the enteric epithelium are not represented.

Fig. 7.—Section a little distance in front of the anterior end of the ovary with the posterior parts of the second pair of nephridia ($n.^2$), the vitelline glands and the obliquely running longitudinal bands of cœlenchyme.

Fig. 8.—Section through the anterior part of the ovary with the loops of the third pair of nephridia (n^3.) on the dorsal side of the ovary and the ventral side of the stomach.

Fig. 9.—Section immediately following that represented in fig. 8, with vertical septa enclosing a median space in which are the nerve-cord and the bends of the loops of the third pair of nephridia ($n.^3$), which almost meet in the middle line.

Fig. 10.—Section passing through about the middle of the large ovum.

Fig. 11.—Section passing through one of the cirri of the third pair, and on the other side passing through the funnel or cushion ($cu.$) and the shell-gland secretion ($sh.$) in the neighbourhood of the genital aperture.

Fig. 12.—Section a little further back comprising the thickenings of the septum ($s.^2$) behind the large ovum, shell-gland secretion ($sh.$) and the genital aperture (♀).

PLATE 13.

Fig. 13.—Transverse (slightly oblique) section through the head region at the bases of the anterior limbs. × 600.

Fig. 14.—Part of a transverse section from another series passing through the external genital aperture (♀), shell-glands ($sh. gld.$), ciliated cushion ($cu.$), with the large ovum ($o.^1$) and the thickening of the septum ($s.^2$) behind it. × 500.

Fig. 15.—Section through the caudal region showing the relations of the cœlenchyme. × 600.

Fig. 16.—Anterior plates of lower jaw in S. tasmanicus. × 2400

Fig. 17.—Anterior plates of lower jaw of S. novæ-hollandiæ. × 2400.

Fig. 18.—Cirrus of posterior limb of S. novæ-hollandiæ.

Fig. 19.—Cirrus of posterior limb of S. tasmanicus.

Figs. 20–22.—Penis in S. novæ-hollandiæ.

Figs. 23–25.—Penis in S. tasmanicus.

PLATE 14.

Fig. 26.—Rami of upper jaw in S. novæ-hollandiæ partly everted with the parts slightly separated.   × 2400.

Fig. 27.—Ramus of upper jaw of the same incompletely retracted, showing relations to lower jaw and bridle (represented by dotted out-lines).   × 2400.

Fig. 28.—Transverse section of longitudinal muscular fibres of Stratiodrilus showing the relations to the cœlenchyme.   × 1500.

Fig. 29.—Longitudinal muscular fibres of an Enchytræid (from a transverse section) with muscle nuclei and a peritoneal nucleus ; c = cœlomic epithelium plus the myocyte layer.   × 1500.

Metameric Segmentation and Homology.

By

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With Plates 15 and 16.

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IN two recently published papers on the development of the fins of fish and on the segmentation of the head of Amphibia (**12, 15**), I had occasion to discuss incidentally the segmental relations of homologous organs, and to point out that, in the Vertebrates at all events, corresponding parts must be considered as fully homologous although occupying different segments of the body. In this paper I shall not attempt to define the nature of segmentation nor trace its origin, but shall merely try to show that a practical definition of the homology of an organ must not depend on its position in the series of segments. The subject of metameric segmentation has been very clearly dealt with by Sir E. Ray Lankester in articles on the Arthropoda and Metamerism in the tenth and eleventh editions of the 'Encyclopædia Britannica' (reprinted in vol. 47 of this journal). While giving a comprehensive review of the whole question of metamerism, he states thirteen "laws," or general propositions, with most of which what follows will be found in complete agreement. But, in spite of the results of Fürbringer (**9, 10**) from anatomical investigations, of Bateson (**1**) from observations on variation, and of others, there is, I think, a reluctance on the part of many anatomists to give up the idea that true homology depends on segmental correspondence. For instance, when discussing

the homology of the occipital condyle in the Amphibia and
the Amniota, we are asked on what segments they occur, it
being implied, if not expressly stated, that if they are not on
the same segment they cannot be homologous.

The whole subject of homology and segmentation is very
complex, imperfectly understood, and well worthy of further
study. In the following paper I have endeavoured to put the
theory clearly before the reader, and to come to some definite
conclusion.

Let us consider first of all the paired limbs. No one will
deny that the pectoral limbs are homologous throughout the
land-vertebrates or Tetrapoda ; and the same may be said of
the pelvic limbs. By homologous we mean, in this case, that
these organs can be traced back in an uninterrupted phyletic
series to some common ancestral form. Yet the limbs do not
necessarily occupy the same segments. For instance, in the
Amphibia, while the pectoral limb belongs to segments 2, 3
and 4, in the Anura, it belongs to segments 2, 3, 4 and 5, in
the Urodela ; and while the pelvic limb occupies segments 8, 9
and 10, in the frog, it is in segments 16, 17 and 18 in
Salamandra, in segments 20, 21 and 22 in Necturus, and still
farther back in Amphiuma. Moreover, the sacral vertebra is
the ninth in the frog, the sixteenth in Salamandra, and the
twentieth in Necturus (see Diagram 1).

Among the living Reptilia the limbs vary considerably in
position, especially the pelvic (Diagram 1). For instance,
while in Chamæleo the brachial nerve-plexus extends over
segments 3–6, and the pelvic plexus over segments 18–22,
in Lacerta the former is from segment 6 to 9, and the
latter from segment 26–31. In the limbless reptiles the
nerve-plexus can still be found (Gadow (11A) Carson (5)).
Thus Amphisbæna has a brachial plexus in segments 2, 3 and
4, and a pelvic plexus in segments 97, 98 and 99 ; Bungarus,
a brachial plexus in segments 4 and 5, and a pelvic in segments
230 and 231 ; Python has a brachial plexus in the third and
fourth segments, and a pelvic in segments 342–345. The
sacral vertebræ are the twenty-fifth and twenty-sixth in

Stellio; the twenty-seventh and twenty-eighth in Ascala-
botes; the twenty-ninth and thirtieth in Lacerta; the sixty-
fifth and sixty-sixth in Seps. In birds also we find a remark-
able inconstancy in the relative position of the limbs [for an
admirable and detailed study of this question see Fürbringer's
great monograph, (**10**)]. To take only three examples : in
Cypselus the brachial plexus extends over segments 10–14,
and the pelvic over segments 20–27 ; in Podiceps the brachial
plexus occupies segments 15–18, and the pelvic segments 26–
35; while Struthio has a brachial plexus in segments 17–21,
and a pelvic plexus in segments 29–38. (see Diagram 1).

Even the Mammalia show considerable variation in the
position of the pelvic limb, although that of the pectoral limb
remains remarkably constant. The lumbo-sacral plexus of the
rat includes the lumbar nerves 2–6, of the guinea-pig the
lumbar nerves 3–6 and first sacral, of the gnu the lumbar
nerves 4–6 and first and second sacral [Paterson (**18**)].

No less conspicuous is the shifting of the paired fins in
fishes, as will be easily seen on consulting Diagrams 2
and 3.

In the foregoing account the genetic relation of the limbs
to the segments has been determined by the nerve-supply in
the adult. This is amply justified by a study of the development,
which shows that any change of position during ontogeny,
caused by the shifting of the limb-rudiment from its place of
origin, is insignificant. The limb is not formed on one set of
segments, and subsequently transferred to another region and
supplied by new nerves. On the contrary, limbs arise in the
embryo in that region which they occupy in the adult. As a
rule several segments contribute to the formation of each
limb, the skeletal tissue musculature and nerves being derived
from these segments. When, as in many fish, the radial
muscles of the paired fins are formed from buds of the myo-
tomes, their exact relation to the segments can be accurately
made out. " Concentration," or the relative narrowing of the
base of attachment of the fin, may introduce secondary
modifications, which do not, however, affect the position of

the limb as a whole (**12**). The nerves supplying the radial muscles are branches of the spinal nerves corresponding to the myotomes from which the muscle-buds were developed; and the course of these nerves in the adult necessarily indicates the source of the fin muscles they supply, while their size is proportional to the amount of muscle derived from each particular segment. This is the case even when neighbouring muscular segments fuse to compound muscles, as usually happens in paired limbs.[1] The branches of the segmental spinal nerves may combine more or less completely to form a complex limb-plexus, but they nevertheless preserve their original connections with the spinal cord, and also their original peripheral connections with the muscle-substance derived from their own segments. It follows that the nerve-supply in the adult is a safe guide in deciding both which segments have been concerned in the formation of the limb, and in what proportion they have contributed to it.

We must conclude from these facts that the paired limbs do not bear a constant relation to any given segments in the Vertebrates, that they appear to shift along the segmented trunk, that this apparent shifting has taken place independently and repeatedly in the different classes, and that it can take place either forwards or backwards. For in the modern Anura the pelvic limb is almost undoubtedly farther forwards than in the ancestral Amphibian, and in the teleostean fish the more recent and modified forms may have the pelvic fin far forwards, until in extreme cases it reaches a position immediately behind the pectoral.

Not only do the paired limbs shift their position, but they also vary greatly in their extent, being sometimes formed by many and at other times by only a few segments. In the fishes this variation is extraordinary, and in Tetrapods it is less pronounced, but still considerable.

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[1] In my paper on Fins (**12**) I maintained, against the views of Braus and others, that the radial muscles preserved the original segmentation. But this is not the case, and the error was corrected in a subsequent paper (**15**).

Or to put the matter in a more general way—suppose we call the consecutive segments A B C D . . . In one case the limb may belong to segments A B, in another to segments B C, and in a third to segments C D, and so on. Again in one animal a limb may belong to segments E F, in another to segments C D E F G, and in a third to segments A B C D E F G H. How are we to reconcile these facts with our conception of homology?

Some anatomists seem to consider the paired limbs and limb-girdles as special organs developed outside the truly segmented region of the body, as it were independent of it, and therefore capable of moving from one region to another. But this view is quite inadmissible; as already pointed out above, it is directly contradicted by the well-established facts of anatomy and embryology. The limbs are as truly segmented as the myotomes and vertebral column, and are formed from the same materials. The segmentation may, of course, be obscured in the adult, just as it may be in the trunk or head, but it is obvious in development. Moreover, the limbs never really abandon the segments from which they originated (12). It certainly is not by a theory of the migration of ready-formed limb-material in the embryo that we can explain the shifting of limbs, or preserve our ideal of homology.

Migration during ontogeny may indeed occur, but to a very limited extent. The direction and amount of the migration is faithfully recorded in the adult by the course of the nerves supplying the limb. For instance in the frog the straining backwards of the pelvic plexus shows that the base of the hind limb was moved back with the elongation of the pelvic girdle. In such fish as the whiting, where the pelvic fins have attained a jugular position actually in front of the pectorals, their real place of origin is betrayed by the nerves which pass backwards to the spinal cord, crossing those supplying the pectoral fins (see Diagram 4).

Certain variations in the attachment of the pelvic girdle to the sacral vertebræ may be explained in the same way. In the salamander, as shown by von Jhering (17), the pelvic

limb is normally supplied by the sixteenth, seventeenth and eighteenth spinal nerves, and the girdle is attached to the sixteenth vertebra. Occasionally, however, while the plexus remains in the same position the sacral vertebra is the seventeenth. Thus the limb and girdle, which presumably developed from the same segments in the two cases, become connected with the vertebral column one segment farther back, their real place of origin being shown by the nerves. It is only such slight secondary shiftings that the theory of migration during ontogeny can explain.

There remain three other possible explanations of the varying position of limbs to be considered : the theory of intercalation and excalation, the theory of re-division, and the theory of progressive modification or transposition.

The theory of intercalation has been admirably dealt with by von Jhering in a classical memoir (17). According to this view, the relative shifting of a limb and its plexus along a series of segments in different animals is explained on the supposition that one or more segments have dropped out or new segments have been added between pre-existing segments. This process is taken to account not only for the apparent motion of a structure up or down the segmental series, its change of position, but also for the extension of a limb or plexus over more or fewer segments than it originally occupied. It is necessary to suppose that segments can be intercalated or excalated at any point in front of a plexus, within the plexus, or behind it.

Von Jhering first deals with such simple cases as Sorex, where a whole segment seems to have been intercalated in front, some individuals having thirteen and others fourteen dorsal vertebræ, the whole pelvic plexus and sacrum in the latter being situated one segment farther back. Or, again, Cynocephalus, in which the last lumbar vertebra and its nerve appear to have dropped out, leaving the plexus reduced by one nerve, and the pre-sacral vertebræ twenty-five instead of twenty-six in number. At first sight the theory seems to afford a plausible explanation of such variations. Less easy

is it to explain the next set of cases, in which the plexus is modified in extent or position without an accompanying change in the vertebral column.   For instance, in the rabbit the lumbo-sacral nerve-plexus may in some individuals include numbers 25–30 and in others numbers 24–30, while the first sacral vertebra is the twenty-seventh in both cases.   Here a nerve seems to have been intercalated in the middle of the plexus, since the crural nerve (coming chiefly from 26 and 27 in the first case) appears to have moved one segment forwards, the ischiadic remaining behind in its original position.

To account for these changes, von Jhering has to make another assumption : it is that the nervous system being derived from the epiblast and the skeleton and muscles from the mesoblast, they can vary independently, and that a segment having been intercalated or excalated in the series of nerves, a re-arrangement takes place so that the plexus fits again on to the unaltered vertebral column in a new position.   The nerves slip a cog, so to speak, and catch on in a new place. The whole theory becomes very artificial.   By the dropping out now of nervous segments, and now of skeletal segments, and again of complete segments comprising both elements, it seems possible to explain almost any conceivable variation in the disposition of these parts.   But what evidence is there to support these assumptions ? none whatever.

If the difficulties are great in the way of explaining the varying position of the limbs in these simple cases, they are formidable indeed when applied to those forms in which the differences are more pronounced, as, for instance, in the fishes.   The smelt, Osmerus eperlanus, has a pectoral fin supplied by the spinal nerves 1–4, and a pelvic supplied by nerves 18–29 (Hammarsten, **16**), while in the whiting, Gadus merlangus, the nerves 1–4, supply the pectoral fin, and nerves 5–6 the pelvic (Diagram 3).   Again, in Scymnus the pectorals are supplied from nerves 2–13, and the pelvics from nerves 23–35; in Torpedo nerves 4–30 supply the pectoral, and nerves 31–42 the pelvics (Diagram 2).   If the

approximation of the pelvic fin to the pectoral had been brought about by the excalation of segments, we should have to assume that the whole trunk region between the two had been suppressed in Gadus and Torpedo—an obviously absurd assumption, as Fürbringer has already pointed out. Moreover, twenty-seven new segments would have to be intercalated in the region of the pectoral fin of Torpedo. Now since such vast differences frequently occur between closely allied forms, we should have to suppose that this wholesale appearance or disappearance of segments takes place rapidly and repeatedly. If such were really the case, should we not expect to find some indication of the process—zones of growth or zones of reduction, where new segments would be forming, or old ones vanishing? No trace of such zones is found, either in the adult or in the embryo.[1] On the contrary, segments are found to grow only at the extreme end of

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[1] It may, of course, be suggested that segments can be intercalated and excalated in the course of phylogeny just as they appear to be in ontogeny. But the two processes must be clearly distinguished. First of all there is, I believe, no evidence that segments are ever intercalated in ontogeny. Observation seems to have established that new segments are only formed at the growing end of the series. On the other hand, it is obvious that segments may be more or less completely crushed out in ontogeny—as, for instance, in the occipital region (see below). But it must be remembered, however, that these segments are, as a rule, clearly formed in early stages of development, and that our inability to make them out in later stages is mainly due to practical difficulties of observation and technique. The work is done on a series of individuals killed at various stages of development; often the series is incomplete and the stages separated by wide gaps. Could we observe one individual continuously, we should probably be able to trace out the fate of each segment with certainty. As a matter of fact the somites of the occipital region are generally distinct enough in the early stages of the most modified Craniates. Still, it may be supposed that if the process of reduction began earlier and earlier in successive generations, the crushing out might at last take place at the very beginning of growth, and that these segments might finally cease to appear at all as such. It would be rash to say that this has never happened; but, so far as I know, there is no convincing evidence that it has ever been the fate of segments in the Vertebrata.

the series.[1] It is difficult to see how some 300 segments could
be intercalated in the trunk of a snake such as python
without the formation of some zone of growth ; yet no such
zone is present.   But it may be answered that the appearance
in some specimens of incompletely double vertebræ shows
how new segments could be added one by one.   Such
occasional abnormalities, however, do not necessarily indicate
the mode of formation of new segments ; they can be more
easily explained as due to partial fusion and incomplete
development, or as pathological phenomena induced by early
injury.

We have seen to what absurd conclusions the theory of
intercalation would lead us if applied to the vertebrates as
an explanation of the variation in position of the paired limbs
and girdles.   The whole theory is based on too narrow and
rigid a conception of homology.   It assumes that a structure
A is definitely and unalterably related to a given segment x,
which can be traced from one animal to another ; that
wherever A is found there also must be x.   When A changes
its position it is then necessary to suppose, for the sake of
preserving its strict homology, that x has moved with it.
This results in the paradox that to preserve the homology of
the limb, we are obliged to sacrifice the homology of whole
regions—in some cases of nearly the whole trunk.   And even
then the strict homology of the limb (say the pectoral of
Torpedo) is not really saved.   The sacrifice has been all in vain,
for new segments have been added to it (seventeen in the case
of Torpedo as compared with Scymnus ; see Diagram 2).   It is
really quite futile to attempt to define the homology of an
organ in a segmented body by its ordinal position in the series
of segments.[2]

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[1] Segments appear to be always added at the posterior end ; but they
may be retarded in their development at the anterior end, as in the case
of the head somites of the Craniate.

[2] From the point of view of a study of variation Bateson rightly
maintains "the impossibility of applying a scheme of homology
between individual segments " p. 128 (1).

Further, the theory cannot afford even a formal explanation of the change of position of the median fins relative to the paired fins in fishes, as I have already pointed out elsewhere (12). The median fins in Elasmobranchs are similar in structure and development to the paired fins, and, like these, shift up and down the body in different forms. Now the interesting and quite conclusive fact to notice is that the two sets of fins shift independently. For instance, the first dorsal fin is opposite the pectoral in Lamna, between the pectoral and the pelvic in Alopecias, opposite the pelvic in Scyllium, and behind the pelvic in Raja. If it is granted that the fins are homologous in these four genera, no addition or suppression of segments can possibly account for their disposition. Other cases of homologous organs passing in front or behind one another could be given.

The evidence against the theory of intercalation is overwhelming. Moreover, it undermines the very foundation of the definition of homology it is intended to uphold; for if segments can be added to or removed from any part of the series, there is no guarantee that any one particular segment in one individual really corresponds to any particular segment in another individual.

Let us now briefly examine a view which may be called the theory of re-division. It may be stated as follows : if one individual is composed of, say, twenty segments, and another of twenty-one, 'the difference is due, not to the addition of a new segment, but to the subdivision of the individual in the first case into twenty segments and in the second case into twenty-one segments. Therefore, no segment of the first individual can be strictly homologous with any segment of the second. If the number of segments is sufficiently increased by further sub-division into twenty-two, twenty-three, and so on, an organ originally situated on, say, the fifth segment might later on be found on the sixth or seventh ; and two organs originally close together might become separated by a larger and larger number of segments. Although this theory may appear promising at first sight, it soon becomes obvious that it could only apply to the

very simplest cases, and is quite incapable of accounting for the relative shifting of organs accompanied by the unequal variation of segments in different regions. Whether we measure an object in inches or in centimetres the relative position of its parts remains unaltered. Only could there be relative shifting if the redivision was unequal along the series. Comparing, for instance, the vertebral column of Dromæus with that of Struthio, we might say that in the former the cervical region has been divided into eighteen and the lumbar region into six segments, while in the ostrich the cervical region has been divided into seventeen and the lumbar into eight segments. Obviously this would be no explanation at all, but merely a statement in different words of the original problem we set out to solve. No doubt such a view avoids the difficulty of zones of growth or of reduction; but no more than the theory of intercalation can it be applied to such cases as the apparent suppression of the mid-region of the trunk in fishes where the pectoral meets the pelvic fin, or the independent shifting of the median and paired fins discussed above. Some theory of the redistribution of the formative substances to which morphological differentiation is due is necessary if we are to explain homology; mere redivision does not help us at all.[1]

If neither migration nor intercalation nor redivision can account for the change in position of fins or paired limbs, there remains the theory of transposition (**12** and **13**). Fürbringer has shown how the nerve-plexus of a limb may become more or less extensive by the gradual assimilation of the nerves of neighbouring segments (**9**). Nerves at the anterior or posterior end may increase in size, and new nerves from adjoining segments may enter into the composition of a plexus; so that by gradual growth a limb originally supplied by, say, nerves E F G, may come to be supplied by nerves D E F G H, C D E F G H J, and so on. Or, on the contrary, by a similar

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[1] It may well be doubted whether "redivision" ever takes place. When the total number of segments varies, the variation may better be interpreted as due to differences in growth—that is to say, to the addition or suppression of segments at the growing end of the series.

but reverse process of reduction, a limb originally supplied by
nerves c–j may come to be supplied only by nerves e f g.
If such were really the case we should expect to find the limits
of a plexus somewhat indefinite and variable, the more
important and stouter nerves towards the middle, and slenderer
twigs contributed by nerves at each end. Now this is just
what anatomical investigation reveals; a limb-plexus is built
on this plan.

Further, a limb-plexus may shift, without altering its general
structure, from one region to another, by such a process of
growth or extension at one end accompanied by reduction at
the other end. New segments being assimilated along the
direction of growth, others may drop out, ceasing to contribute
to the plexus. Thus a nerve-plexus may successively occupy
segments d e f, e f g, f g h, and g h j. By a gradual
process a plexus comes to occupy an entirely new position,
having been transposed without the appearance or disappear-
ance of any segments at all. What is true of the nerves is
doubtless true also of the musculature and skeletal elements
(Diagram 5). The fact that the position of a nerve-plexus and
the size of its component nerves is an accurate guide to the
segmental composition of a limb has already been sufficiently
dwelt upon above.

This theory of the shifting of a plexus and a limb from one
region to another is in complete harmony with the teaching
of comparative anatomy and embryology. It is the only
theory which gives a reasonable explanation of such cases as
Raja and Gadus, and the independent motion of the paired
and unpaired fins mentioned above. The process whereby
the transposition[1] is brought about may be interpreted as due
neither to the insertion or removal of segments nor to the

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[1] To the correspondence between two similar sets of nerves forming a
plexus, but occupying different ordinal positions in the series of
segments, Fürbringer has applied the word Parhomology. This term,
however, seems to imply that the homology is incomplete, whereas it is
here contended that it may be as complete as between any two organs.
Fürbringer also applies the term "imitative homodynamy" to the

migration of parts after the segmentation has been laid down in ontogeny, but to a redistribution at some very early stage in the unsegmented embryo of the formative substances.

It is quite clear, then, that the conception of homology in a segmented vertebrate is independent of any consideration of the number or ordinal position of the segments which compose the parts under comparison. If organs to be called homologous have to be composed of the same segments, we should have to conclude that the pectoral limbs of birds and reptiles are not homologous—which is absurd. It is merely an unnecessary complication to introduce the idea of segmental correspondence into a definition of homology. The heart, the liver or the lungs are held to be fully homologous throughout the Craniata, quite irrespective of any possible relation to segments ; in the same way the pectoral limb of a reptile and of a bird may be called homologous merely as being corresponding parts of common origin. And so it is with other parts of the body ; the various regions of the vertebral column, in so far as possessed by a common ancestor, are homologous, but they are not necessarily composed of the same segments.

Let us now consider the case of the occipital condyles, which seems to present greater difficulties. There is abundant evidence among the Pisces that the posterior limit of the head is variable, that more segments are assimilated into the head region in some fish than in others, that by growth and differentiation, by a process of transposition strictly comparable to that of fins, the hind limit of the head moves up or down the segmental series. More or fewer segments become included in the occipital region, more or fewer gill-slits develop and are supplied by a corresponding number of branches of the compound vagus nerve. Transposition can account for all these changes.

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assimilation of one segment to another when it takes on the form and function of another. Bateson calls this homœosis (1), but it seems to me difficult to apply this term to the transposition of a structure which occupies one segment only.

But the interpretation of the occipital region is complicated by the well-established fact that one or more myotomes immediately behind the auditory capsule may undergo degeneration during ontogeny. These are the myotomes corresponding to the glosso-pharyngeal, the first, and the subsequent vagus dorsal roots, and supplied by the ventral spino-occipital roots. They tend to disappear in development from before backwards, and their nerves go with them. It might be thought that here at last we have a case of excalation.[1] Indeed, Fürbringer himself, if I understand him rightly, seems to believe that the most posterior spino-occipital nerve, z, shifts forwards by the disappearance in phylogeny of the more anterior roots s, t, u, etc.

The process of ontogenetic degeneration of myotomes in the occipital region must not be confused with excalation. The glosso-pharyngeal supplying the first branchial slit, and the first root of the vagus supplying the second slit, form a well-differentiated nerve complex which can be homologised throughout the craniate vertebrates. If the shifting of the hind limit of the head is to be attributed to the disappearance of segments behind the auditory capsule, then it is clear that (unless we sacrifice the homology of the glosso-pharyngeal and first vagus and their slits for the sake of saving that of the occipital segment) we must suppose that the segments have vanished, not at the anterior end of the metaotic series, but in the middle region—that is to say, between the first vagus root and the condyle. This supposition is directly against the evidence of embryology, which plainly shows that the more anterior segments are more degenerate than the posterior. We should also be compelled to assume that the change of position of the occipital condyle, being due to the disappearance of segments, could take place in one direction only—towards the anterior end. It would follow that the forms with an occipital region containing few segments have always been derived from those with many, which is in the highest degree improbable.

[1] See footnote, p. 241.

As in the case of the limbs, the theories of intercalation or redivision fail utterly when applied to the occipital condyles. The segmental homology of the condyles cannot be saved by such means without sacrificing the homology of other structures of equal importance.

Turning now from these general considerations to the comparison of the condyles in the Amphibia and Amniota (15). There appear to be not more than three mesoblastic segments between the occipital condyle and the auditory capsule in the amphibian, and not less than five in the amniote.[1] If the condyle is on the same segment in the two groups, two segments must have disappeared in Amphibia. These cannot have vanished at the front end of the series, as already pointed out above, unless the glosso-pharyngeal and vagus nerves, etc., are new in the amphibian—not homologous with those of the amniote—a supposition which is obviously not admissible. There is absolutely no evidence in embryology that the segments have disappeared between the vagus and the condyle ; but for the sake of argument let us suppose that they have. Does this help us out of our difficulties? Certainly not ! On the contrary it lands us in a worse position than before. For it must then be supposed that the fourth branchial slit of the amphibian corresponds to a sixth of the amniote, and the whole homology of the branchial arches and other connected structures is upset. And we are further met with an insuperable difficulty concerning the hypoglossal

[1] The results of most authors who have studied the composition of the occipital region of the Amniota are remarkably uniform. Van Bemmelen (3), Corning (6) and Sewertzoff (19) in reptiles. Froriep (7), Belogolowy (2), in birds, find four myotomes in the embryo. The first is quite vestigial and belongs to the second meta-otic somite, since the first somite corresponding to the glosso-pharyngeal nerve is much reduced and never forms muscle (see Diagram 6). In mammals there appear to be also five somites and three distinct myotomes (Froriep (8) and others). A varying number of ventral nerve-roots join to form the hypoglossal. The first spinal (or post-occipital) still contributes to it in the reptiles and birds, but in the mammalia the hypoglossal roots are entirely intra-cranial.

nerve. This nerve, or complex of nerves, belongs with the
various parts it supplies to a region extending in the
Amphibia behind the occipital condyle to the second spinal
nerve (the hypoglossal in the frog comes out between the
first and second vertebræ—see Diagram 6)—and in front of
the first vertebra in the Amniota.[1] The independent trans-
position of skeletal and neuro-muscular elements can alone
account for such relative displacement.

Of course the whole question of the homology of the
condyles in the Amphibia and Amniota may be avoided by
supposing that they have been independently developed in
the two groups, are not homologous, and have not altered
their position since they made their first appearance. But,
although we are not able to prove with absolute certainty
that the common ancestor already possessed a skull with
differentiated occipital region and well-defined condyles, yet
the evidence points strongly to this conclusion. The reptiles
and stegocephalous Amphibia merge into each other in
the Permian and Carboniferous strata, and for my own part, I
am firmly convinced that they are all derived from some
common terrestrial ancestor with well-developed condyles.
However, even supposing this was not the case, we should
still be met by the difficulty of the hypoglossal. And since
the common ancestor cannot be held to have been provided
with several condyles, one of which remains in the amphibian
and the other in the amniote, the theory of transposition
seems to be the only one applicable in this case, as it has
already been shown to be in the case of the fins.

The conclusion to which we are driven is that the occipital
condyles of amphibians, reptiles, birds and mammals are all
homologous, whatever may be the segments on which they are
developed. They are fully homologous in the only sense in
which homology can be practically defined, namely in the
sense that they can be traced back to a common ancestor. In
the course of evolution the function of condyle-formation,
originally belonging to segment n of the series, has been trans-
posed to segments $n + 7$ or $n - 7$. The transposition of the

nerves and other parts may or may not have accompanied that of the condyles.

Thus a consideration of the condyles, like that of the regions of the vertebral column or limbs, inevitably leads us to the conclusion that homology is independent of ordinal correspondence in segmental position. Specialised organs or any differentiated parts are truly homologous in different animals when they are derived from corresponding parts in the common ancestor—the animals being compared as wholes and not their separate segments. And this is true whether these organs or parts are composed of few or of many, of the same or of different segments, or are not segmented at all.

On the other hand, any attempt to define homology as complete only when including strict segmental correspondence defeats its own object. It is doubtful whether any organs or parts whatsoever could be proved to be completely homologous in this sense; certainly not the paired limbs, nor the regions of the vertebral column, nor the occipital condyles. Even when homologous organs appear to be composed of the same segments, the appearance may be deceptive; for if any form of multiplication or of intercalation of segments can take place, the comparison of segment with segment becomes at once uncertain.

To advocate the view that homology must be considered as independent of segmental correspondence is the object of this paper; but in conclusion a few words may be said concerning the possible connection between them. It is a remarkable fact that a constant relation often becomes established between certain segments and certain organs or differentiated parts.

The various cranial nerves, for instance, appear to bear a fixed relation to the anterior segments of the head throughout the Craniata. The muscles of the eye, the ear, the jaws, the gill-slits, etc., are all definitely related to certain segments, more or less closely and in different ways. The neck of mammals (with but three exceptions) is formed of seven segments, the last lumbar vertebra of the Artiodactyle Ungulates is always the twenty-sixth, the vertebral column of the

Teleostean family Triacanthidæ is composed of twenty verte-
bræ, and so on. Undoubtedly a correspondence of this kind
may become established, but it appears to be of secondary im-
portance. Generally speaking it is more definite and invariable
in the anterior than in the posterior region, and in regions or
animals composed of few than in those composed of many
segments. It is just as if Nature got tired of counting towards
the tail end of a developing animal, and as if her arithmetic
became uncertain when dealing with large numbers. But
structure and segmentation vary independently, and whatever
may be the connection which becomes established between
them, and however close it may be, it would seem that we
must not consider it as constant and essential.

In the segmented vertebrate the materials for the formation
of muscular nervous and skeletal segments are distributed
along the body; those particular segments which occur in ap-
propriate positions are entrusted, so to speak, with the develop-
ment of special organs. The function of producing an organ
may be transposed from one segment to another. How this
transposition is brought about we do not know as yet; pos-
sibly it is accompanied by the redistribution of organ-forming
substances at a very early stage, regulated by a complex of
stimuli subordinated to the needs of the individual as a whole.[1]

[1] What has been said of the Vertebrates would probably apply equally
well to the Annelids or Arthropods. Although their segmentation may
very well have been quite independently developed, yet it resembles
that of the vertebrate in that segments appear to be always increased
or diminished in number at the posterior end of the series. The same
specialisation of segments, of appendages, and of other repeated parts
takes place, and the same apparent shifting. In these also it is to be
explained by transposition. Any appropriate segment in the series
may be called upon to develop a particular kind of appendage or a
genital duct, for instance, and the number of these organs may be
increased or diminished without a corresponding change in the whole
number of segments. We may speak of a general homology of the
several series of repeated parts, and of a more special homology of any
one of them which, like the mandible or green gland or oviduct, may be
traced to a common ancestor. Just as in the vertebrate, so in the
annelid and arthropod, a very constant relation may be established

But then, it will be objected, is homology to be reduced to
a vague and almost meaningless comparison ? Not at all !
Comparing a horse with a man, we may say that the trunk of
the one is generally homologous with the trunk of the other ;
further, that the fore limb of the horse is homologous with the
fore limb of man ; and further still, that the one digit of the
former is more specially homologous with the middle digit of
the latter, and so on to the minutest detail—to the smallest
blood-vessel, to a single nerve-fibre, even to a single cell.
The homology may be perfectly definite, and pursued to the
furthest conceivable limit. For it to be recognised it is only
necessary that the structures compared should, at all events
in theory, be traced to a common ancestral origin.

The completeness of the homology depends on how far all
the parts can be so traced back. The homology is impaired by
the addition or loss of any parts. For instance, the pectoral
girdle of the Teleostome fish is incompletely homologous with
that of the Elasmobranch, since dermal bones of different
origin have been added in the Teleostome to the primitive
endoskeletal girdle, and it is incompletely homologous with
the pectoral girdle of the mammal, because the latter has
almost or entirely lost the dermal elements. Two organs are
completely homologous when all their parts have been derived
from corresponding parts in the common ancestor.

Summary.

In the Vertebrates, as in other animals, the organs and parts
of two individuals are to be considered as homologous when
they can be traced back to corresponding parts in a common
ancestor, and not because they occur on the same segments.

between these organs and segments of a certain numerical order ; and
this relation is also more often found in animals composed of few
segments than of many, and at the anterior than at the posterior end
of the body (see further the articles on Arthropoda and Metamerism
written by Sir E. R. Lankester in the 'Encycl. Brit.,' eleventh edition,
and reprinted in this journal, vol. 47).

The homology is independent of the number and ordinal position of the segments which take a share in the formation of the organs. Any structure may apparently shift from one segment to another; and this is brought about neither by intercalation or excalation of segments, nor by redivision, nor by migration, but by a process of transposition. Organs may be homologous when they are composed of few or of many, of the same or of different segments, or are not segmented at all. There are degrees of homology: it may be general or more special, complete or incomplete. The homology of two organs is complete when all their parts have been derived from corresponding parts in a common ancestor.

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EXPLANATION OF PLATES 15 and 16,

Illustrating Mr. Edwin S. Goodrich's paper, 'Metameric
Segmentation and Homology.'

PLATES 15 and 16.

Diagram 1.—The position of the paired limbs as shown by the nerve-
supply. The line o represents the hind limit of the head. The spinal
nerves contributing to the limb plexus are numbered. The range of
the pectoral plexus is covered by a continuous line, that of the pelvic
plexus by a broken line. S shows the position of the sacral vertebræ.

Diagram 2.—The position and extent of the paired fins of Torpedo
and Scymnus as shown by the nerve-supply (from the observations of
Braus). A continuous line covers the pectoral plexus and a broken line
the pelvic plexus.

Diagram 3.—The position and extent of the paired fins of Osmerus
eperlanus (after Hammarsten) and of Gadus merlangus. The first
nerve in the former and the first two nerves in the latter pass through
the skull.

Diagram 4.—The nerve-supply of the paired fins of Gadus mer-
langus: A in their natural position, and B with the pelvic brought
back to its place of origin.

Diagram 5.—Three figures illustrating the principle of transposition as seen in the development of median fins. From the myotomes m are derived the radial fin-muscles $r.m.$ 1–11 branches of spinal nerves.

Diagram 6.—The head-region of an Amphibian A, and an Amniote (mammal) B. The broken line o–o indicates the hind limit of the occipital region. 1–7 myotomes. $a.$ Auditory capsule. $br.s.$ Bronchial slit. $f.$ Facial nerve. $gl.$ Glosso-pharyngeal. $h.$ Hypoglossal. $h.m.$ Hypoglossal musculature. $n.a.$ Neural arch. $n.c.$ Nasal capsule. $o.p.$ Optic capsule. $s.$ Tympanum. $s.g.$ Vestigial spinal ganglion. $s.o.$ First metaotic somite. $sp.$ Spinal nerve. $tr^1.$ Profundus. tr^2 Trigeminal. $v.$ vagus nerve.

The Metaphase Spindle in the Spermatogenetic Mitoses of Forficula Auricularia.

By

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With Plate 17.

INTRODUCTION.

In a paper published at the beginning of this year I have shown that only one generalisation has been established concerning the mitotic spindle, viz. that it is not a figure formed entirely by the action of forces at its poles. The arguments put forward by Hartog in support of his " mitokinetic " force prove that, if this generalisation is denied, the spindle can be formed by no known forces. Gallardo and Rhumbler have been compelled to accept this proposition; and all early theories either admit it, or are disproved because they do not admit it.

I pointed out that in the circumstances we must collect data, and not attempt to explain mitosis until other generalisations have been established. Our knowledge of the morphological features of the cell and of the chemical nature of its component parts is rudimentary ; and, since we know nothing concerning the forces working in mitosis and concerning the changes undergone by the mechanism in the course of evolution, speculation upon the subject is at present likely to prove abortive. The work of each decade has shown more and more that the cell is a highly complex entity ; and the results of future research may prove it to be even more complex than we now suspect. Recently several writers upon both chromosomes and the achromatic portions of the cell have suggested

that chemistry will provide the solution of problems that so far we have failed to solve. That eventually we must hand over to others problems that are found to lie beyond the scope of our investigations cannot be denied; but, until we have amassed data such that we can answer every question concerning the morphology and movements of structures that are visible in the cell, we are not justified in relying upon other branches of science for more than co-operation in our endeavour to explain these phenomena.

In a paper upon chromosome dimensions published last year I was able to show that increasing somatic complexity of the organism is accompanied by increase of the volume of chromatin in its germ-cell, and that the diameter of chromosomes becomes greater as we pass from low to higher phyla of the animal kingdom. I now propose to carry out similar investigations upon the phenomenon known as the mitotic spindle, and shall try to discover whether at a given moment in a given mitosis the length is constant or arbitrary; if it is found to be constant, we must determine the relationships between lengths at corresponding stages of successive mitoses, and must ask if these relationships are connected with those of other phenomena.

The stage that is most easy to identify in mitosis is the conclusion of the metaphase, when the chromosomes have undergone complete fission, and the daughter-rods, apposed to one another, are ready to move towards the two poles. I shall therefore make consideration of this stage the basis of my research, and shall determine the length of the spindle by measuring the distance between the centrosomes. I have chosen Forficula for these investigations, because the chromosomes are either spheres or very short rods; it is accordingly easy to recognise the moment at the conclusion of the metaphase when constriction is completed. Since the two centrosomes can seldom be brought into focus simultaneously at the highest magnifications, figures in which the spindle length is to be measured will be represented in the plates by two drawings : the first will show a lateral view of the equatorial

plate at a magnification such that no doubt can exist concerning the stage of mitosis; the second will show a lateral view of the two centrosomes at a lower magnification, from which the length of the spindle must be deduced.

It is unlikely that these measurements will lead directly to an explanation of spindle formation; but they may suggest some further generalisation, which will bring us one step nearer to the solution of this problem.

Material and Methods.

The material was collected in July, and preserved in either Flemming's strong chromo-aceto-osmic acid fluid or the platino-aceto-osmic acid solution of Hermann. In an earlier paper I recommended the latter; but for these studies, in which extreme transparency of the cytoplasm is essential, the former has undoubtedly given the better results.

The testes were not dissected out until required for embedding; but the integument of the back was slit open to ensure immediate access of the fixative, in which the material remained for twenty-four or forty-eight hours. It was then washed in running water for twenty-four hours, and passed successively through 30 per cent., 50 per cent. and 70 per cent. aqueous solutions of alcohol, remaining for four hours in each of the two first-named and for eight hours in the last. It was then stored in a solution of 80 per cent. alcohol.

Later, the testes were placed in a 90 per cent. aqueous solution of alcohol for twenty-four hours, and then passed through a 95 per cent. solution, absolute alcohol, and xylol; after which they were embedded in paraffin having a melting-point of 52° C. Sections were cut 8μ thick with an ordinary Cambridge rocking microtome, and were stained on the slide. The mordant used was an aqueous solution of iron alum, in which the sections remained for six hours; they were then stained for fifteen hours in Heidenhain's iron-hæmatoxylin, and the excess of colour was later washed out

with a weak solution of the iron-alum. When a plasma stain was used in conjunction with the iron hæmatoxylin, the slides were first stained for ten minutes in eosin, care being taken that the colour was not destroyed by the strong solutions of alcohol in which they were later placed.

The preparations were studied by means of a Zeiss apochromatic oil-immersion objective of 2 mm. focus and N. A. 1·30, in conjunction with compensating oculars, Nos. 6, 12 and 18. When necessary, resolution was facilitated by interposing a Gifford screen. The source of illumination was an inverted incandescent gas-lamp, used in combination with the holoscopic oil-immersion substage condenser made by Messrs. Watson & Sons, of London. All drawings were made with a large Abbe camera lucida, and the magnification was estimated by means of a stage micrometer, graduated to read one hundredth of a millimetre. Whenever drawings were about to be made, both microscope platform and drawing table were carefully levelled; and error due to foreshortening was obviated by making drawings only of those spindles whose major axes lay exactly at right angles to the microscopic line of vision, i. e. whose centrosomes could be focussed simultaneously. Moreover, in order to minimise error due to draughtsmanship, the centrosomes of each spindle were drawn upon several occasions, and at least one hundred times in all.

The Length of the Mitotic Spindle at the Conclusion of the Secondary Spermatocyte Metaphase.

Figs. 1 to 5, Pl. 17, are drawings of polar views of the secondary spermatocyte metaphase, and all the chromosomes are shown. Those chromosomes that are short rods lie on the spindle in a manner such that their major axes are at right angles to the equatorial plane; consequently all must appear to be spherical in a perfectly polar view. The complex is composed of a single ring of ten chromosomes with two lying within it, or of a ring of nine chromosomes with three within it; the former arrangement is seen in

figs. 1, 4 and 5, and the latter in figs. 2 and 3. I have failed to discover which arrangement is normal.

Fig. 6, Pl. 17, is a lateral view of the equatorial plate in a late secondary spermatocyte metaphase, and the bivalent chromosomes are seen to be undergoing constriction. This stage therefore immediately precedes that with which we propose to deal. The centrosomes of this cell are shown at a magnification of 889 diameters in fig. 49, Pl. 17, and the distance between them, estimated from this magnification, is 7.8μ. Fig. 7, Pl. 17, shows the equatorial plate at the conclusion of the metaphase; constriction is here seen to be complete, and the dyads have become resolved into pairs of univalent chromosomes, which are ready to pass towards the two poles. Fig. 50, Pl. 17, shows the centrosomes of this cell, and the amount of their divergence is found from the drawing to be 8.1μ. Figs. 8, 9 and 10, Pl. 17, are drawings of the equatorial plate in three cells undergoing successive stages of the anaphase, and the daughter-chromosomes are observed to be moving further and further apart. The centrosomes of these cells are represented in figs. 51, 52 and 53 respectively, and the distances between them, estimated from the known magnification, are 8.3, 8.5 and 8.7μ.

Consideration of the drawings of these five cells suggests that the length of the spindle at the moment when constriction of the chromosomes is complete is a constant for this mitosis of the individual. It is reasonable to suppose that, if fixation had been delayed until the centrosomes of fig. 6 were 8.1μ apart, the appearance of the chromosomes would have been similar in every respect to that seen in fig. 7; and we must likewise suppose that the equatorial plate in figs. 8, 9 and 10 was identical with that of fig. 7, when the length of the spindle in these three cells was 8.1μ. We will, however, check these results by making drawings of a second set of five cells in which the distances between the centrosomes are respectively 7.8, 8.1, 8.3, 8.5 and 8.7μ. Figs. 11 to 15, Pl. 17, are lateral views of the equatorial plate of these cells, and each is seen to correspond exactly with the drawing

immediately above it. Constriction of the chromosomes is in progress in fig. 11, and is completed in fig. 12; in figs. 13, 14 and 15 the daughter-chromosomes appear to be moving further and further apart. The centrosomes of these cells are represented by figs. 54 to 58 respectively; and these drawings, made at a magnification of 889 diameters, are respectively identical with those given in figs. 49 to 53. These measurements, which have been made from cells belonging to the testes of a single specimen, can leave little doubt that the length of the spindle at the conclusion of the metaphase is a constant for this cell generation of the individual.

We must now ask if this constant may be assumed for all members of the species. Figs. 16 to 20, Pl. 17, are drawings of the equatorial plate of five cells in the testes of a second specimen, and each is seen to correspond exactly with the two drawings placed immediately above it. Fig. 16 shows a late metaphase, which is found to be concluded in fig. 17; figs. 18, 19 and 20 represent successive stages of the anaphase. Figs. 59 to 63, Pl. 17, show the centrosomes of these cells, drawn at a magnification of 889 diameters. And, since these drawings are respectively identical with figs. 49 to 53 and 54 to 58, we have reason for believing that the spindle length is a constant at the conclusion of the secondary spermatocyte metaphase in all specimens of F. auricularia.

The Length of the Mitotic Spindle at the Conclusion of the Primary Spermatocyte Metaphase.

Having discovered a probable constant for the spindle of the secondary spermatocyte metaphase, we will consider the primary spermatocyte mitosis. Figs. 21 to 24 represent polar views of the metaphase, and all the chromosomes are shown; fig. 25 is a drawing of the slightly later stage when the daughter-chromosomes have begun to move towards the poles. As in the secondary spermatocyte metaphase, the major axes of those chromosomes that are short rods are at right angles to the

equatorial plane. The complex again appears to be composed
of a ring of nine or ten chromosomes with three or two respec-
tively lying within it; figs. 21, 22 and 25 represent the latter
arrangement, and figs. 23 and 24 the former. I have again
failed to discover which arrangement is normal.

Figs. 26 to 29, Pl. 17, are drawings of the equatoral plate of
four cells of which the centrosomes, represented at a magni-
fication of 889 diameters, are given in figs. 64 to 67, Pl. 17. The
drawings on Pl. 17 clearly show that constriction of the tetrads
has been completed, and that the daughter-chromosomes are
ready to move apart. The length of the spindle, found from
figs. 64 to 67, is without exception $10.4\,\mu$; and, since the
stage of the cells depicted is that with which we are dealing,
we have reason for supposing that a constant exists also for this
mitosis. The uneven pair of heterochromosomes is marked
x in those cells in which it is visible.

Let us now measure the spindle length in four more cells in
order to test the validity of this supposition. Figs. 30 to 33,
Pl. 17, are drawings of the equatorial plate in cells of which
the centrosomes are respectively represented by figs. 68 to 71,
Pl. 17. Fig. 30 shows the constriction of the tetrads in pro-
gress; fig. 31 shows this constriction completed, as in figs. 26
to 29; and figs. 32 and 33 show the first divergence of the
daughter-dyads. The distances between the poles of these
cells, found from figs. 68 to 71, are respectively 10.2, 10.4, 10.7
and $10.9\,\mu$, and therefore accord with the length of the spindle
found for figs. 26 to 29. We must suppose that fig. 30 would
have become identical with fig. 31, if fixation had not occurred
until its centrosomes were $10.4\,\mu$ apart; and we must likewise
suppose that the equatorial plates shown in figs. 32 and 33
passed through the stage shown in fig. 31, when their spindles
were of the same length as that of the last named. In the
circumstances I shall assume that the length of the spindle at
the conclusion of the primary spermatocyte metaphase is a
constant for this species.

I have found measurements in this metaphase more difficult
to make than in that of the secondary spermatocyte; for

spindles often appear to be distorted, and the distances between the poles may consequently be greater than those shown. The fact that no such distortion is observed in the spermatogonial and secondary spermatocyte mitoses suggests that the large size of the primary spermatocyte cells renders section-cutting, however carefully carried out, destructive of the true form of the spindle in certain cases. Such cells, however, may be recognised, because the spindle-fibres appear to be abnormally bent and twisted, and are often disconnected from the centrosomes. After careful consideration I am therefore satisfied that the measurements given represent accurately the dimensions within the cell.

The Length of the Mitotic Spindle at the Conclusion of the Secondary Spermatogonial Metaphase.

We will now consider the spermatogonial mitosis, and try to discover if a constant exists also for this metaphase. Figs. 34 to 37, Pl. 17, are drawings of polar views of this metaphase, showing all the chromosomes. Fig. 38 represents a polar view of one daughter-plate in the earliest anaphase. The complex appears to be composed of two concentric rings of fourteen and eight chromosomes respectively, while two chromosomes lie at the centre; this is the arrangement seen in figs. 34, 35, 37 and 38. In fig. 36, thirteen chromosomes constitute the outer ring, and nine the inner; but this arrangement may have resulted from abnormal movement caused by the process of section-cutting. The chromosomes that are short rods lie on the spindle in a manner such that their major axes are parallel to the equatorial plane, and thus differ in position from those of the spermatocyte metaphases.

Fig. 39, Pl. 17, is a drawing representing a lateral view of the equatorial plate before constriction of the bivalent chromosomes has begun. The centrosomes of this cell are shown in fig. 72, and the length of the spindle is found from this drawing to be $6·6 \mu$. Fig. 40 is a lateral view of the equatorial plate at a slightly later stage, when the dyads have begun to

constrict; in this case the distance between the centrosomes is found from fig. 73 to be 6·9 μ.   Fig. 41 represents the equatorial plate at the conclusion of the metaphase, and the dyads are seen to have become resolved into pairs of univalent spheres or rods, which are ready to move towards the poles.   Fig. 74 shows the centrosomes of this cell, and the length of the spindle, estimated from the known magnification, is 7·1 μ. Figs. 42 and 43 are drawings of the equatorial plate in the earliest anaphase, and the daughter-chromosomes are seen to be moving apart.   The centrosomes of these cells are represented in figs. 75 and 76 respectively, and their distances apart are 7·3 and 7·6 μ.   It is difficult not to believe that figs. 39 and 40 would have resembled fig. 41, if fixation had not taken place until their spindle-poles were 7·1 μ apart ; it is also difficult not to believe that figs. 42 and 43 were identical with fig. 41 at the moment when this was the distance between their poles.

Let us now turn to cells of this generation in the testes of another specimen.   Fig. 44 shows the equatorial plate before constriction of the chromosomes has begun ; figs. 45 and 46 show the equatorial plate while constriction is in progress ; and figs. 47 and 48 show it at the conclusion of the metaphase, when constriction is completed.   The centrosomes of these five cells are represented in figs. 77 to 81 respectively, and their distances apart accord with those already observed for this mitosis; for the length of the spindle is found to be 6·6 μ in fig. 44, 6·9 μ in figs. 45 and 46, and 7·1 μ in figs. 47 and 48. In the circumstances I shall assume that the length of the spindle at the conclusion of the metaphase is a constant for the secondary spermatogonial mitosis, as we have already assumed it to be for the primary and secondary spermatocyte.

The Ratios between the Lengths of the Mitotic Spindle at the Conclusion of the Spermatogonial and Spermatocyte Metaphases.

Having found that spindle lengths are probably constant at the conclusion of the metaphase of these mitoses, we

must ask if the relationships between the lengths observed can be correlated with those of other phenomena.

Now the length of the spindle is evidently not connected with the volume of chromatin in the equatorial plane. I have shown in an earlier paper that the volume of chromatin in the metaphase is the same in the spermatogonial and primary spermatocyte mitoses; whereas it is reduced to one half in the secondary spermatocyte. If correlation is to be established, we must accordingly find the same spindle length in the metaphase of the two first-named mitoses, and a different spindle length in the last. We find, however, that the length of the spindle is not the same in any two of these metaphases. Furthermore, it is evident that the spindle length is not connected with the number of chromosomes present; for the number of chromosomes composing the spermatogonial complex is double that composing the complexes of the primary and secondary spermatocytes. The length of the spindle at the conclusion of the metaphase cannot therefore be correlated with the chromatin of the cell.

Let us now ask if the spindle length can be correlated with the cytoplasm. We know that no growth or resting stage occurs between the primary and secondary spermatocyte divisions, and that consequently the volume of the cell in the second must be half that in the first. Now the ratio between the radii of two spheres of which the volume of one is equal to twice that of the other is 1·26 : 1·00. And this is almost exactly the ratio between the lengths of the spindle found for these two metaphases; for the lengths found are 10·4 and 8·1 μ, and—

$$10\cdot4 \; : \; 8\cdot1 \; :: \; 1\cdot28 \; : \; 1\cdot00.$$

If, therefore, inaccuracy of measurement is responsible for the slight difference between these ratios, the length of the spindle at the conclusion of each spermatocyte metaphase seems to be proportional to the radius of a sphere equal in volume to the cell.

We will now consider the secondary spermatogonial spindle. The length found for this mitosis at the stage with which we

are dealing is $7 \cdot 1 \, \mu$; and the ratio between this length and that observed at the corresponding stage of the primary spermato-cyte is $1 \cdot 00 : 1 \cdot 46$. Now we do not know the ratio between the volumes of the secondary spermatogonial and primary spermatocyte cells in the metaphase; for a long period of growth intervenes. But, if the spindle length at the conclusion of the spermatogonial metaphase is correlated with the cell volume, as we have reason for supposing that it is correlated in the two succeeding mitoses, we can determine the ratio between the volumes of the secondary spermatogonial and spermatocyte cells at this stage. Let us assume this. Now, the ratio between the radii of two spheres of which the volume of one is equal to three times that of the other is $1 \cdot 44 : 1 \cdot 00$, and this is almost identical with the ratio between the lengths of the spindle at the conclusion of the primary spermatocyte and spermatogonial metaphases; for these lengths have been found to be $10 \cdot 4$ and $7 \cdot 1 \mu$, and we have already seen that—

$$10 \cdot 4 \ : \ 7 \cdot 1 \ :: \ 1 \cdot 46 \ : \ 1 \cdot 00$$

The difference between these two ratios is so small that it may be ignored; and, if our assumption concerning the relationship between the spindle length and cell volume in the metaphase is correct, we must realise that the volume of the primary spermatocyte cell at this stage is equal to three times that of the secondary spermatogonial. But the initial volume of the former must be half that of the latter, because the secondary spermatogonium divides to form two daughter primary spermatocytes. The volume of each primary spermatocyte must therefore be increased six-fold during the growth period. I have already shown by actual measurement that the volume of the chromatin is doubled during this period; and, since a large increase in the cell volume is always apparent at its conclusion, the possible connection between the length of the spindle and the volume of the cell in the spermatocyte metaphases may be extended to the spermatogonial mitosis.

CONCLUSION.

In the introduction of this paper I have said that the measurements to be made in the course of these investigations may suggest a further generalisation in the problem of mitosis. We have now made these measurements, and have found that they do suggest a new generalisation, viz. that the length of the mitotic spindle at the conclusion of the metaphase is proportional to the radius of a sphere equal in volume to the cell.

The measurements upon which this proposition is based have been made with great care; but it is possible that coincidence is responsible for the connection found between the spindle length and cell volume in the spermatocytes, and for the apparent connection in the spermatogonia. If, however, coincidence is not responsible, correlation is established between these phenomena in the spermatogenetic metaphases of this species. I intend in subsequent papers to measure spindle lengths at corresponding stages in other organisms; and, if the same relationships are found, I hope that my results will be corroborated by those of other cytologists.

SUMMARY.

(1) The length of the mitotic spindle, i. e. the distance between the two centrosomes, at the stage of the metaphase when the chromosomes are undergoing constriction in the equatorial plane, appears to be a constant for each spermatogenetic mitosis of the species. The lengths found are 6·9, 10·2 and 7·8 μ for the secondary spermatogonia and primary and secondary spermatocytes respectively.

(2) The length of the mitotic spindle at the conclusion of the metaphase when the daughter-chromosomes are ready to move apart appears to be a constant for each spermatogenetic mitosis of the species. The lengths found are 7·1, 10·4 and 8·1 μ for the secondary spermatogonia and primary and secondary spermatocytes respectively.

(3) The length of the mitotic spindle in the earliest

anaphase, i. e. at the moment when the daughter-chromosomes have begun to move apart, appears to be a constant for each spermatogenetic mitosis of the species.   The lengths found are 7·3, 10·7 and 8·3 μ for the secondary spermatogonia and primary and secondary spermatocytes respectively.

(4) The ratio between the lengths of the mitotic spindle at the conclusion of the primary and secondary spermatocyte metaphases is almost identical with the ratio between the radii of two spheres of which the volume of one is equal to twice that of the other ; and the volume of the primary spermatocyte cell must be equal to twice that of the secondary spermatocyte at this stage, because no growth or resting stage intervenes.

(5) The ratio between the lengths of the mitotic spindle at the conclusion of the primary spermatocyte and secondary spermatogonial metaphases is almost identical with the ratio between the radii of two spheres of which the volume of one is equal to three times that of the other.   The initial volume of the primary spermatocyte cell must be half that of the secondary spermatogonium, because the latter divides to form two daughter primary spermatocytes ; but the large size of the last-named, observed at the close of the growth period, does not refute the suggestion that the initial volume is increased six-fold during this period.

(6) If coincidence is not responsible for the apparent connection between the ratios mentioned above, correlation is established between the cell volume and length of spindle in the spermatogenetic metaphases of this species.

March, 1913.

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N.B. : A comprehensive list of publications dealing with mitotic phenomena is given in the bibliography of my paper, " The Problem of Mitosis."

EXPLANATION OF PLATE 17,

Illustrating Mr. C. F. U. Meek's paper, " The Metaphase Spindle in the Spermatogenetic Mitoses of Forficula Auricularia."

Figs. 1–4.—Polar views of secondary spermatocyte metaphase, showing all chromosomes. Specimen A.

Fig. 5.—Ditto. Specimen C.

Fig. 6.—Lateral view of equatorial plate in late secondary spermatocyte metaphase, showing constriction of bivalent chromosomes. Specimen A.

Fig. 7.—Lateral view of equatorial plate at conclusion of secondary spermatocyte metaphase, showing univalent daughter-chromosomes resulting from completed constriction of dyads. Specimen A.

Fig. 8.—Lateral view of equatorial plate in earliest secondary spermatocyte anaphase, showing divergence of daughter-chromosomes. Specimen A.

Fig. 9.—Ditto, later. Specimen A.

Fig. 10.—Ditto, later. Specimen A.

Fig. 11.—Lateral view of equatorial plate in late secondary spermatocyte metaphase, corresponding with fig. 6. Specimen A.

Fig. 12.—Lateral view of equatorial plate at conclusion of secondary spermatocyte metaphase, corresponding with fig. 7. Specimen A.

Fig. 13.—Lateral view of equatorial plate in earliest secondary spermatocyte anaphase, corresponding with fig. 8. Specimen A.

Fig. 14.—Ditto, later, corresponding with fig. 9.   Specimen A.

Fig. 15.—Ditto, later, corresponding with fig. 10.   Specimen A.

Fig. 16.—Lateral view of equatorial plate in late secondary spermatocyte metaphase, corresponding with figs. 6 and 11.   Specimen B.

Fig. 17.—Lateral view of equatorial plate at conclusion of secondary spermatocyte metaphase, corresponding with figs. 7 and 12. Specimen B.

Fig. 18.—Lateral view of equatorial plate in earliest secondary spermatocyte anaphase, corresponding with figs. 8 and 13.   Specimen B.

Fig. 19.—Ditto, later, corresponding with figs. 9 and 14.   Specimen B.

Fig. 20.—Ditto, later, corresponding with figs. 10 and 15.   Specimen B.

Fig. 21.—Polar view of primary spermatocyte metaphase, showing all chromosomes.   Specimen D.

Figs. 22 to 24.—Ditto.   Specimen A.

Fig. 25.—Polar view of equatorial plate in earliest primary spermatocyte anaphase.   Specimen B.

Figs. 26-29.—Lateral views of equatorial plate at conclusion of primary spermatocyte metaphase, showing daughter-dyads resulting from completed constriction of tetrads.   The unequal pair of heterochromosomes is marked x.   Specimen A.

Fig. 30.—Lateral view of equatorial plate in late primary spermatocyte metaphase, showing constriction of tetrads.   Specimen A.

Fig. 31.—Lateral view of equatorial plate at conclusion of primary spermatocyte metaphase, corresponding with figs 26-29.   The unequal pair of heterochromosomes is marked x.   Specimen A.

Fig. 32.—Lateral view of equatorial plate in earliest primary spermatocyte anaphase, showing divergence of daughter-dyads.   Specimen A.

Fig. 33.—Ditto, later.   Specimen A.

Figs. 34-37.—Polar views of spermatogonial metaphase, showing all chromosomes.   Specimen D.

Fig. 38.—Polar view of earliest spermatogonial anaphase.   Specimen B.

Fig. 39.—Lateral view of equatorial plate in early spermatogonial metaphase, before constriction of bivalent chromosomes has begun. Specimen D.

Fig. 40.—Lateral view of equatorial plate in late spermatogonial metaphase, showing constriction of dyads.   Specimen D.

Fig. 41.—Lateral view of equatorial plate at conclusion of spermatogonial metaphase, showing univalent daughter-chromosomes resulting from completed constriction of dyads.   Specimen D.

Fig. 42.—Lateral view of equatorial plate in earliest spermatogonial anaphase, showing divergence of daughter-chromosomes. Specimen D.

Fig. 43.—Ditto, later. Specimen D.

Fig. 44.—Lateral view of equatorial plate in earliest spermatogonial metaphase, corresponding with fig. 39. Specimen B.

Figs. 45, 46.—Lateral view of equatorial plate in late spermatogonial metaphase, corresponding with fig. 40.

Figs. 47, 48.—Lateral view of equatorial plate at conclusion of spermatogonial metaphase, corresponding with fig. 41. Specimen B.

Fig. 49.—Centrosomes belonging to equatorial plate depicted in fig 6. Estimated divergence in cell, 7·8 μ.

Fig. 50.—Ditto in fig. 7. Ditto, 8·1 μ.

Fig. 51.—Ditto in fig. 8. Ditto, 8·3 μ.

Fig. 52.—Ditto in fig. 9. Ditto, 8·5 μ.

Fig. 53.—Ditto in fig. 10. Ditto, 8·7 μ.

Fig. 54.—Ditto in fig. 11. Ditto, 7·8 μ.

Fig. 55.—Ditto in fig. 12. Ditto, 8·1 μ.

Fig. 56.—Ditto in fig. 13. Ditto, 8·3 μ.

Fig. 57.—Ditto in fig. 14. Ditto, 8·5 μ.

Fig. 58.—Ditto in fig. 15. Ditto, 8·7 μ.

Fig. 59.—Ditto in fig. 16. Ditto, 7·8 μ.

Fig. 60.—Ditto in fig. 17. Ditto, 8·1 μ.

Fig. 61.—Ditto in fig. 18. Ditto, 8·3 μ.

Fig. 62.—Ditto in fig. 19. Ditto, 8·5 μ.

Fig. 63.—Ditto in fig. 20. Ditto, 8·7 μ.

Figs. 64–67.—Ditto in figs. 26–29 respectively. Ditto, 10·4 μ.

Fig. 68.—Ditto in fig. 30. Ditto, 10·2 μ.

Fig. 69.—Ditto in fig. 31. Ditto, 10·4 μ.

Fig. 70.—Ditto in fig. 32. Ditto, 10·7 μ.

Fig. 71.—Ditto in fig. 33. Ditto, 10·9 μ.

Fig. 72.—Ditto in fig. 39. Ditto, 6·6 μ.

Fig. 73.—Ditto in fig. 40. Ditto, 6·9 μ.

Fig. 74.—Ditto in fig. 41. Ditto, 7·1 μ.

Fig. 75.—Ditto in fig. 42. Ditto, 7·3 μ.

Fig. 76.—Ditto in fig. 43. Ditto, 7·6 μ.

Fig. 77.—Ditto in fig. 44. Ditto, 6·6 μ.

Figs. 78, 79.—Ditto in figs. 45 and 46. Ditto, 6·9 μ.

Figs. 80, 81.—Ditto in figs. 47 and 48. Ditto, 7·1 μ.

Fig. 82.—Divisions of stage micrometer, 10 μ apart, drawn at same magnification as figs. 49–81. The magnification of these figures is estimated from this to be 889 diameters.

N.B.: Specimen A—preserved in fixative of Flemming, and stained with iron-hæmatoxylin. Specimen B—preserved in fixative of Hermann and stained with iron-hæmatoxylin. Specimen C—preserved in fixative of Flemming, and stained with iron-hæmatoxylin and eosin. Specimen D—preserved in fixative of Flemming and stained with iron-hæmatoxylin.

Studies in the Experimental Analysis of Sex.

Part 10.—The Effect of Sacculina on the Storage of Fat and Glycogen, and on the Formation of Pigment by its Host.

By

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It has been shown in previous studies that Sacculina elaborates fatty material in its roots at the expense of its host, and it has been stated that the livers of infected crabs show a more constantly abundant supply of fat in the liver than normal crabs. Since the maturing ovary of the normal female crab also stores a fatty material in the form of yolk, and since at this time also the female shows an abundant supply of fat in the liver, and, moreover, exhibits characteristic changes in the blood, which becomes loaded with yellow lipochrome (lutein) and fatty material, the suggestion was advanced that the Sacculina roots played the same part in the metabolism of infected crabs as is normally played by the ovary of a maturing female crab, and that the alterations in the secondary sexual characters of infected crabs followed as a consequence of this fat-forming activity of the Sacculina roots.

It is proposed in this study to attempt to get a clearer conception of the activity of the Sacculina roots and of the effect they exert on the storage of food material in the host by the use of two chief methods : firstly, by employing certain micro-chemical reagents for the detection of different fat combina-

tions and of glycogen ; secondly, by quantitative chemical
methods for the estimation of the proportions of fat and
glycogen present in the livers of normal and of infected crabs.
The histological work was done in conjunction with Mr. J. J.
Conybeare, B.A., New College, Oxford, in Dr. Scott's labora-
tory, and we are indebted to Dr. Scott for much help.   In the
quantitative chemical estimations of fat and glycogen, Dr.
Ramsden has given me much invaluable advice, and some of
the earlier experiments were done in his laboratory.   The
rest of the work was carried on at the Marine Biological
Laboratory at Plymouth, and I am indebted to the Director
and staff for their constant attention to my needs.

At the end will be found a summary of the physiological
action of Sacculina on its host.

1. Micro-chemical Methods.

Besides ordinary fat stains, e. g. scarlet R. and Sudān 3,
two principal micro-chemical methods have been used for
detecting and distinguishing fat—the Nile blue sulphate
method and Weigert's method.   These two methods are not
only useful for detecting fat, but they also throw light on the
nature of the fat or lipoid concerned, as has been shown by
Professor J. Lorrain-Smith and W. Mair (" Fats and Lipoids
in Relation to Methods of Staining," 'Skandinavischen
Archiv für Physiologie,' Bd. xxv, 1912.   See this paper for
details of method and reference to literature).

In the Nile blue sulphate method, sections of the tissue
(preserved in formalin 6 per cent.) are cut on the freezing
microtome and stained overnight in a saturated watery solu-
tion of the stain.   These are differentiated in 2 per cent. acetic
acid, washed in water and mounted in gum.   By this method
neutral fat is stained red, fatty acid blue, while a mixture of
neutral fat and fatty acid is stained purple of various degrees
according to the amount of the two constituents present.   The
rationale of this stain, as explained by Lorrain-Smith and
Mair, is " that in the watery solution of the stain there is a

combination of two kinds of dye, one the original basic oxazine dye which unites with fatty acid to form a blue compound, and the other a derivative of the base, a red oxazone not basic in character, but soluble in liquid fat and giving it a red colour."

In Weigert's bichromate hæmatoxylin method sections preserved in formalin and cut on the freezing microtome are subjected to the action of saturated potassium bichromate for periods varying from 12–120 hours at a temperature of about 37° C. They are then washed and stained in hæmatoxylin overnight and subsequently differentiated in Weigert's borax ferricyanide solution.

The staining of the fat or lipoid by the hæmatoxylin depends on the formation of a compound of the fat with chromium oxide which forms a lake with the hæmatoxylin. This compound is, however, only formed by fats in which an unsaturated grouping of the component atoms occurs, and the more of such unsaturated groupings are present the quicker does the bichromate form the compound which is stained by the hæmatoxylin. The length of time, therefore, that the sections have to remain in the bichromate is a measure of the degree to which the fats or lipoids present are saturated or unsaturated.

The employment of the Nile blue sulphate method has given results of more importance for our inquiry than the Weigert method.

We may consider first the proof that the ova of the normal female crab in the course of becoming ripe take up fat from the blood, first of all in the form of neutral fat-globules, and then convert them into yolk-globules with a different staining reaction.

Sections of a practically mature yellow or dark pink ovary stained with the Nile Blue method will show principally ripe eggs containing large yolk-globules which are stained purple. A small cytoplasmic zone round the nucleus is stained blue ; the nucleus is colourless except for a very dark blue nucleolus. In a less ripe ovary eggs are seen which, in addition to the purple staining yolk-globules, contain much smaller globules

in a peripheral cytoplasmic area, and these globules stain bright red, showing that they consist of neutral fat. If an ovary be taken at the period when it has recently started to form yolk—a period which can be recognised by the pale-yellow colour of the ovary—the eggs can be detected in a very interesting period of their growth. On one side of the eggs small globules can be seen, lying in a pale-blue cyto-plasmic area, which stain bright red, while on the other side of the egg these red-staining globules pass through a tran-sitional zone into purple-stained yolk-globules of much larger size. The nucleus, with its colourless nucleoplasm, dark-blue nucleolus and surrounding area of blue cytoplasm, divides the two sorts of globules. As the egg increases in size, the purple yolk-globules go on growing at the expense of the red fat-globules, until the latter are confined to a small peripheral area surrounding the egg, and to some extent are to be seen in the cytoplasmic area round the nucleus. Quite young eggs which have not begun to store yolk consist merely of the nucleus surrounded by a blue-staining granular cytoplasm. It is clear from the observation of these stages that the young eggs take up fat from the blood, and deposit it at first in the form of neutral fat (red-staining globules), and that these neutral fat-globules are worked up into the purple-staining vitellin or yolk-globules probably by the action of the cytoplasm in the region of the nucleus.

In one case, as the result of a pathological change, the reverse of this process was observed, viz. the breaking-down of the yolk-globules into neutral fat again. This was found in a female infected with a Sacculina which had died before coming to the exterior. The ovary of this female consisted partly of very young eggs in process of growth, and partly of degenerating masses of eggs which had nearly reached maturity, but in which the yolk-globules were being broken down to form masses of red-staining neutral fat. In certain places this process of degeneration had gone so far that nothing remained of the eggs except accumulations of neutral fat.

It must be remarked that in the crustacean ovary there are no nurse-cells which elaborate the yolk and pass it on to the eggs ; the latter, on the contrary, are bathed in the blood and take up the fat directly from it.

The Weigert method of staining the eggs did not shed any important additional light on the nature of the yolk-globules. After treatment with the bichromate for eighteen hours, the yolk-globules were intensely stained with the hæmotoxylin after treatment with potassium ferricyanide. It can only be said that the yolk-globules are acted on by the bichromate more rapidly than the neutral fat-globules in the liver.

The treatment of frozen sections of crab's liver and of sacculina roots with Nile Blue gives very clear pictures of the abundant presence of fat in the liver-cells and in the cells of the Sacculina roots. The cytoplasm and nuclei of the cells in both cases stain blue, while the large neutral fat-droplets in the liver-cells are picked out in brilliant red, and the smaller fat-droplets in the Sacculina roots are stained with a magenta tinge. The slight difference in the red colour of the liver fat and of the Sacculina fat probably indicates a slight difference in chemical composition between them, but it is not nearly so marked as the difference between the neutral fat of the liver and the yolk-globules of the mature ova. The Weigert method also indicates a very slight difference in the composition of the fat of the two cases, since the Sacculina fat-droplets are slightly more rapidly acted on by the bichromate than is the case with the liver fat. The difference is, however, so slight that it may merely be due to the smaller size of the fat-globules in the Sacculina roots.

It is possible, therefore, to show by conclusive micro-chemical tests that the Sacculina roots take up fatty material from the blood of the host, which is laid down at first principally, at any rate, as neutral fat. Now, we have seen that the ova in the course of their growth take up fat from the blood in the form of neutral fat, so that the statement made in previous studies that the Sacculina roots are abstracting a

fatty material from the blood similar to that which the ova absorb in a normal female is shown to be accurate.

It is not known in what form the fat is present in the blood of crabs. Although analysis shows fat to be there it is impossible to detect the presence of neutral fat in the undecomposed blood, so that we must presume that it is combined with some other substance in the blood, and is split off by the ova or by the Sacculina roots and deposited as neutral fat within these tissues.

2. On Moulting and the Formation of Pigment.

We have now to examine the effect exerted by Sacculina on pigmentation, moulting, and the glycogen-metabolism connected with moulting.

In previous work (**2** and **3**) Robson and I have shown that the blood of male crabs, especially in the periods leading up to a moult, becomes charged with a pink lipochrome, the destination of which is the skin. It was shown that this pink lipochrome was accompanied with a very small quantity of fat as compared with the yellow lipochrome present in the blood of females with ripening ovaries. Nevertheless, I hazarded the opinion that this pink lipochrome with its accompanying fat was used in the skin as reserve material from which the new skin might in part be formed. If, however, frozen sections of the skin underlying the hard shell of a crab be treated with fat staining reagents, it is found that practically no fat can be detected. Although no fat is present there are large masses of reserve material in the dermis in the form of irregularly shaped refringent clumps which stain intensely with neutral red. These deposits, as is well known, consist of glycogen—a fact which can be most easily demonstrated by extracting with boiling water and adding iodine solution, when the reddish-brown colour characteristic of glycogen is obtained.

Now, if the skin be macerated with strong potash solution and the quantity of ether-soluble substances is estimated, it

is found that although a very rich yield of orange-coloured lipochrome is obtained, yet the percentage in weight of these ether-soluble substances is very small, viz. about 1·5 per cent. Considering the intense coloration of the ethereal solution, it is certain that the greater part of this 1·5 per cent. consists of lipochrome, so that there can be very little actual fat in the skin—a deduction which histological examination amply confirms.

We may conclude, therefore, that fat is not used to any extent as a reserve material for the formation of the new skin, but that lipochrome is employed for the formation of the new skin pigment, and that the principal reserve material for the formation of fresh tissue is glycogen. We can, therefore, readily explain the occurrence of the pink lipochrome in the blood of crabs soon about to moult, and the fact that this pink lipochrome is not accompanied to any great extent with fat, as is the case in the yellow blood of the female at the time of maturity. In her case there is a large mobilisation of fat and of lipochrome for the ovaries, but in the case of the moulting crab only lipochrome is required for the skin and not fat.

Hitherto, in dealing with the lipochrome pigment found in the blood, liver and skin of the crab, we have merely called attention to the existence of two modifications of this pigment —the red, characteristic especially of the blood of males, and the yellow, characteristic especially of the blood of females maturing the ovary. It is convenient at this point to enter more fully into the nature and reactions of these two pigments, and to trace their functions in the organism.

The most illuminating account of the two pigments has been given by Miss Newbigin (4) in her account of the pigments of the salmon, published in the 'Scottish Fishery Reports.'

It appears that the two pigments, the red tetronerythrin and the yellow lutein, which can be abstracted from the muscles and ovary of the salmon, are identical in their reactions with those found in the Crustacea, and that their behaviour in the organism is very similar in the two cases.

Whatever the exact chemical relation between the two pigments may be, and whether or no the one can be converted into the other in the organism, it is clear that the red tetronerythrin is a distinct body from the yellow lutein, and that where the two occur together in an admixture they can be separated by appropriate chemical means. Miss Newbigin has shown that if excess of sodium chloride is added to an extract of salmon muscle made by boiling the muscle with alcohol and caustic soda, a red precipitate is thrown down, while the yellow pigment remains in solution. The red precipitate dissolves in ether to form a yellow fluid, but in absolute alcohol it forms a red fluid. In the dry state it gives a blue colour with nitric or sulphuric acid, and this is considered the typical lipochrome reaction.

The yellow pigment contained in the caustic filtrate differs from the red in several particulars. It does not give the blue lipochrome reaction with nitric or sulphuric acid, so that its inclusion in the category of lipochromes is not strictly correct, though its frequent occurrence with lipochrome and its similar solubility in ether and alcohol have led to its being commonly called a lipochrome pigment. Miss Newbigin remarks, in relation to the yellow pigment : " In the salmon the pigment occurs in the muscle, the ovary, and in large amount in the liver. It is always in close association with fat, and its solubility seems to depend upon that of the associated fat. It does not apparently form compounds with the alkalies or alkaline earths," like the red pigment.

By applying these methods for the separation and detection of the two pigments in the crab, it is possible to make out the following points :

If the shell of a Carcinus is boiled with 60 per cent. caustic potash to which some alcohol is added, a reddish-yellow extract is obtained, and on adding excess of sodium chloride to this, a pink precipitate is formed which gives all the characteristic lipochrome reactions. The filtrate, however, is quite colourless, and does not show the presence of any yellow pigment.

We must conclude from this that all the green and black pigment in the shell of Carcinus is formed from the red lipochrome, tetronerythrin, and that the yellow lutein does not occur in this situation. The fact that the green and black colours in the shell of Carcinus is formed from tetronerythrin in combination with some other substance is shown also in the familiar fact that boiling for some time in water converts all the pigment in the shell to a bright red, and this red pigment can be extracted with alcohol and shown to be tetronerythrin.

The liver of Carcinus yields a very large quantity of the yellow lutein, and either none, or else a mere trace, of the red tetronerythrin. Nevertheless it seems certain that the seat of formation of the tetronerythrin is the liver, since flecks of it can be often detected in this organ, quite distinct from the pale yellow of the general coloration.

In the blood both pigments may occur together, but, as has been already pointed out, a great excess of yellow lutein is characteristic of the maturing female, and a great excess of red tetronerythrin is characteristic of individuals approaching the period of a moult, especially the males. The ovary of the female during its early stages of growth and pigmentation contains almost entirely lutein, but towards the end, when the eggs are ripe, a considerable amount of tetronerythrin is deposited in them as well.

The fact that the adult female in preparing for reproduction is constantly mobilising fatty material accompanied with the yellow lutein, whereas the adult male simply forms tetronerythrin for the skin, gives us a simple explanation of the difference in external coloration which distinguishes the adult males from the adult females in Carcinus. This distinction lies in the fact that the shell of the adult male is always redder than that of the adult female, especially at the joints of the appendages and on the under-surface, and this difference can obviously be accounted for by the excess of red pigment present in the male's blood, and its replacement in the female by the yellow lutein which is destined for the ovary.

It is of considerable interest to note that exactly the same difference between the sexes occurs in the salmon, where the same two pigments are found exercising much the same function as in the crab. The male salmon in the breeding season has a deeper pink skin coloration than the female, which stores up lutein and tetronerythrin in the ovary.

The principal difference between the salmon and the crab lies in the fact that in the salmon the pigments and fats are transferred from the muscles, while in the crab they come directly from the liver.

We must now inquire what effect Sacculina exerts on the formation of these pigments in the crab.

The colour of the shell of infected individuals is of the dull green and brown tint found in females without the bright red characteristic of the male. The blood of Carcinus infected with Sacculina is invariably colourless or else faintly tinged with yellow. The liver, on the other hand, is constantly of a bright yellow colour, and this colour is exhibited with far more constancy than in any other category of crabs, except normal females which are maturing the ovary and have yellow blood. Considerable importance may be attached to this fact, as it indicates that the presence of Sacculina stimulates the liver to the active formation of the yellow lutein. The fact that this substance does not flood the blood of the Sacculinised crabs in the same way that it floods the blood of the maturing female, may be ascribed to the rapidity with which the Sacculina roots seize on the lutein and its accompanying fat and abstract it from the blood.

I have never observed a Sacculinised crab with red blood, and in this respect the observations of Robson on Inachus infected with Sacculina are very puzzling. Robson (3) observed that a very large percentage of Sacculinised Inachus possessed red blood, and though his observations on the livers of infected Inachus agree very well with what occurs in Carcinus, it is very difficult to explain the presence of tetronerythrin in the blood of infected individuals.

If it should prove that this tetronerythrin is only masking

the presence of a large amount of lutein in the blood, or that it only occurs after a period of active lutein formation, the difficulty would be cleared up, and we could confidently say that the condition in Sacculinised Inachus was the same as the condition of normal maturing females, where tetron-erythrin does appear in the blood towards the end of the maturing process.

Unfortunately, we have at present no facts to support this view, and the collection of sufficient quantity of blood from Inachus may prove a severe, though perhaps not insuperable, difficulty in testing the point.

We will leave the question of pigmentation for the present and turn to the question of moulting and the storage of glycogen associated with it.

The deposition of large quantities of glycogen under the skin of crabs as a preparation for the moult has been long known in the case of Decapods, but it can also be shown to occur in Entomostraca, such as Daphnidæ. The late Mr. G. H. Grosvenor put some notes at my disposal on the intra-vitam staining of various Daphnids with neutral red. He found that by keeping Moina for twenty hours in water to which a little neutral red had been added, certain cells in the epidermis became brightly coloured owing to the avidity with which certain refringent bodies contained in their cells took up the stain. On starving a Moina for twenty-four hours and then making it take up neutral red, it was found that these cell-inclusions were greatly reduced in size. From the simi-larity in appearance, situation and staining reaction which these bodies bear to the glycogen deposits in Carcinus, there can be no doubt that they are also composed of glycogen, and represent the material from which the new integument is formed.

The reduction of these reserve deposits during starva-tion is significant, as there is no doubt that in Carcinus also, during starvation, glycogen is removed from the skin and used in the general metabolism, probably after being con-veyed to the liver, since, as will be shown, starvation for a

prolonged period entirely inhibits moulting in Carcinus, but
does not appreciably reduce the amount of glycogen in the
liver.

The influence of Sacculina upon moulting and the de-
position of glycogen in Carcinus is very definite and
of great interest.   After the small Sacculina has once
penetrated to the exterior of its host, the latter never
moults again so long as the Sacculina remains on it, and
even after the Sacculina has dropped off moulting does
not occur for a very long period, and in most cases not at all.
The reason of this inhibition of growth and moulting can be
clearly traced to the inability of the parasitised crab to lay
up sufficient stores of glycogen under the skin, as, if the skin
underlying the hard shell of a parasitised crab be examined,
it is found that a very small amount of glycogen is present
compared to the condition found in a normal healthy crab
with a hard shell.   This cannot be ascribed to a general state
of bad nutrition, as crabs with Sacculina on them always
contain an abundant supply of fat in the liver.   It seems, on
the contrary, that the effect of the Sacculina is to stimulate
the fatty function of the liver and to depress the glycogenic
function, and when we come to consider the quantitative
determinations of fat and glycogen in the liver, we shall find
that this is indeed the true explanation.   It is, however, a
remarkable fact, first noticed by F. A. Potts (5), that Pelto-
gaster, so far from inhibiting moulting in its host, the hermit
crab, actually stimulates it to moult more frequently than
usual—an effect which is the exact opposite of what occurs in
the case of Sacculina and its hosts.

This striking antagonism in results between the two cases
evidently calls for some explanation, and the obvious sugges-
tion to account for it is that the Sacculina and Peltogaster
roots respectively differ in their activities in some way.   It is
clear, for instance, that if the Sacculina roots could be shown
to contain no glycogen, but only fat, while the Peltogaster
roots could be proved to store glycogen in considerable
quantities in addition to fat, then we could readily understand

that Peltogaster would stimulate the glycogenic function of the liver, and lead to an excess of glycogen being formed which could be utilised for the moult, while Sacculina would inhibit glycogen formation and inhibit moulting in conse-quence. Unfortunately this hypothesis will not stand the test of experiment, for sections of the roots of Sacculina and of Peltogaster stained with iodine do not show in either case a trace of the presence of glycogen.

Potts (6) has given a simple mechanical explanation of the reason why infected Pagurids moult while Carcinus and other crabs do not, this explanation being that the Sacculina acts as a mechanical rivet on the comparatively hard tissues of the crab, while Peltogaster, being attached to the soft-bodied abdomen of the Pagurid, does not prevent the old skin from breaking away and being shed. It seems, however, that this can hardly be the true explanation, since the mere presence of a mechanical rivet would not prevent the crab from growing and forming a new skin underneath the old one and of grow-ing until it burst. There must surely be some physiological cause at work in the case of crabs infected with Sacculina which inhibits glycogen storage, growth and moulting.

Whether this cause is active or not in the case of Pagurids infected with Peltogaster we cannot say for certain, but in the next section we shall call attention to certain facts which prove that growth and moulting are not necessarily connected processes, that moulting may take place without growth, and that in this case there is probably a lack of reserve material which prevents growth, although it may not prevent moulting. In the case of Pagurids infected with Peltogaster, it seems certain that the frequent moulting is not accompanied by active growth, because, if it were, infected individuals should on the average be larger than normal, and this is emphatically not the case.

We shall return to this subject after the quantitative results on glycogen formation have been given in the next section.

3. Quantitative Chemical Methods.

We have relied at present upon microscopical investigation for obtaining information as to the relative amount of fat and of glycogen in the livers of normal and infected individuals, but it is clearly desirable to check these results by a more accurate quantitative method. For this purpose the following procedure has been followed, the method for glycogen estimation being Pfluger's (7), and for fat a modification of Leathes' (8). A weighed quantity of liver, 12–20 grm. total wet weight, is obtained from crabs of a particular category, and is boiled on a water-bath for three hours with an equal weight of 60 per cent. caustic potash solution. The resulting mixture is washed out into a beaker with distilled water, and cooled, and to it is added three times the volume of 96 per cent. alcohol, by which the glycogen is precipitated. The precipitate is collected in a Gooch filter, and thoroughly washed with 70 per cent. alcohol. The alcoholic filtrate, which contains the fat, is set apart for further treatment. The glycogen precipitate is dissolved in boiling water, and the glycogen solution so obtained is acidified with hydrochloric acid, so that the strength of the acid in the solution is about 2 per cent. The acidified solution is boiled on a water-bath for two hours to convert the glycogen into sugar. The strength of the sugar solution, which is made up to a known volume, is then estimated by Pavy's method of titrating against copper hydrate solution. In this way the quantity of glycogen in the liver taken can be determined.[1]

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[1] A serious source of error in the titration of the sugar solution with copper hydrate must be guarded against. The solution of glycogen dissolved in hot water always contains some other organic material including amides derived from the breakdown of the proteid. These amides, if present in sufficient quantity, will form a stable greenish compound with the copper hydrate, which prevents the disappearance of the colour on titration and thus destroys the value of the copper as an indicator. This difficulty can be got rid of by evaporating the sugar solution to be tested nearly to dryness on a water bath and re-dissolving in water, after which treatment the action of the amides on the copper hydrate no longer occurs while the reducing power of the sugar is not interfered with. The difficulty only arises in an acute form in testing weak sugar solutions.

The volume of the alcoholic filtrate containing the fat is measured, and a measured portion of it is evaporated on a water-bath until all the alcohol is driven off. The watery residue, containing the fats in the form of soaps, is treated in a long-necked flask with 40 per cent. sulphuric acid, by which the fatty acids are liberated from the soaps. A measured quantity of petroleum ether is added and shaken up with the mixture vigorously for an hour.   A measured portion of the petroleum is pipetted off and evaporated to dryness in a weighed beaker. The beaker containing the solid residue of fatty acid is weighed again, and thus the amount of fatty acid present in the original filtrate can be calculated.

Since the liver of a single crab does not furnish a sufficient quantity of glycogen for estimation, it is necessary to pool the livers of several crabs. For the purpose of the experiments two categories of crabs was used for comparison—normal male crabs with hard skins, and crabs infected with Sacculina. For each experiment livers of four or five normal males were taken and treated together, but with the infected crabs, owing to their smaller size, it was found necessary to take the livers of six to ten individuals for each separate determination.   The percentages given in the following table are therefore each based, not on determinations made on single individuals, but on several taken together, viz. four or five in the case of normal males, and six to ten in the case of infected individuals.

In order to get over the effect of food recently taken and to test the effect of starvation, the crabs were starved for varying periods beginning at 24 hours and going up to 532 hours.

In the table given below the results of glycogen and fat estimation are given in percentages calculated as sugar and fatty acid respectively. In the first column the number of hours during which the crabs were starved is given, and the results of the determinations of glycogen and fat are entered in the other columns opposite, according to the length of time the animals from which the livers were taken were starved.   The

two left-hand columns refer to the normal males, the two on the right to infected individuals.

To take an example. After 72 hours' starvation one lot of normal crabs were estimated and gave 1·01 per cent. glycogen and 9·2 per cent. fat; two lots of infected crabs were estimated after 72 hours, and gave ·44 per cent. glycogen and 13·24 per cent. fat in one case, and ·42 per cent. glycogen and 16·06 per cent. fat in the other.

Table showing Percentages of Glycogen and Fat in the Livers of Normal Male and of Infected Carcinus Mænas.

	Normal males.		Infected individuals.	
Hours of starvation.	Percentage of glycogen, calculated as sugar.	Percentage of fat, calculated as fatty acid.	Percentage of glycogen, calculated as sugar.	Percentage of fat, calculated as fatty acid.
24	1·88	10·59	·99	14·06
	1·1	11·5	·99	12·97
	·65	9·31	·86	16·16
	·36*	5·65*	—	—
48	1·13	13·53	1·09	12·79
	·82	7·65	·81	—
	—	—	·5	9·32
72	1·01	9·2	·44	13·24
	—	—	·42	16·06
96	1·49	11·03	·42	14·79
	·98	—	—	—
	·57	12·32	—	—
168	1·03	9·09	·49	16·34
	·86	11·87	·16	12·48
	·51*	8·63*	—	—
	·4*	5·63*	—	—
288	1·17	11·07	below ·1	12·39
532	·35	13·32	below ·1	12·53
Total percentages	·89	10·02	·56	13·59

* An asterisk signifies that the crabs used for the experiment were soft individuals that had recently moulted.

. The normal males used for the experiments were in all cases, except three, hard-shelled males, which were presumably about mid-way between two moults.

In three experiments (marked with an asterisk in the table) soft-shelled crabs which had recently moulted were chosen, and it will be at once observed what an exceedingly low percentage of fat and of glycogen these crabs possessed. It is clear, therefore, what a pronounced effect the moult has on the storage of food material in the crab's liver, and the greater variability shown by the normal males as compared to the infected individuals as brought out by the table, is attributable in any case to a great extent to the fact that the normal males used were in various degrees of proximity to a moult, whereas the infected individuals were all equally removed from it.

Taking the total average percentages given at the bottom of the table, we find that the normal males on the average have more glycogen and less fat in the liver than infected crabs when starved over a period of 24–532 hours.

Taking the glycogen contents of the liver alone, it can be seen by consulting the second column of figures that starvation for periods up to 532 hours does not have any constant or perceptible effect in reducing the quantity of glycogen in the liver of normal male crabs.   Thus, after 288 hours' starvation the liver contained 1·17 per cent. glycogen, which is an average amount.   Another point is that after only 24 hours' starvation we may observe great variations in the glycogen content, viz. from 1·88 per cent. to ·36 per cent.   This is in keeping with histological results, where the variations in the quantity of glycogen in the liver appears to vary according to the period in the life-history of the crab, especially in relation to the moult.

The infected crabs, whose liver-glycogen content is given in the fourth column, show a marked difference from the normal males.   In the first place, a much more constant percentage of glycogen is present after 24 and 48 hours' starvation, varying only between 1·09 and ·86, and still more conspicuous

is the steady drop in glycogen content after 48 hours' starvation. This indicates that the infected crabs have not any accessory stores of glycogen in the body to draw upon, so that after a certain period of starvation the liver glycogen is called upon to supply the material for carrying on the ordinary metabolic processes of the body, and is consequently soon nearly exhausted. We have already seen that Carcinus infected with Sacculina does not possess stores of glycogen in the skin to anything like the same extent as normal crabs, so that we can easily understand this drainage of glycogen from the liver in the case of infected crabs which does not occur in the case of normal crabs. In the latter it is probable that during starvation the skin glycogen is transferred from the skin to the liver and used for ordinary metabolism—a supposition which is amply confirmed by the fact that starved or underfed crabs never moult in an aquarium however near they may be to the moult when captured.

The quantitative results on glycogen-content of the livers of normal and infected crabs fit in, therefore, very well with the other observations as to the lack of glycogen in the skin of infected crabs and their incapacity to moult.

Turning to the fat percentages, we remark the same variability in the normal crabs as in the case of the glycogen-content. The effect of starvation up to 532 hours appears to be negligible, since there is no regular diminution in fat-content to be observed as the starvation is prolonged, but from 24 to 532 hours' starvation the variation in fat-content runs from 5·65 per cent. to 13·53 per cent.

Comparing this with the fat-content of infected crabs, as exhibited in the fifth column of the table, we see, firstly, that the variability is not so great, and, secondly, that the average percentage in the case of the infected crabs is a good deal higher than in the normal males. It is true that in certain cases the normal males show a fairly high percentage of fat, e. g. 13·53 per cent., but it has never been claimed that in every case infected crabs have a higher percentage than the normal. What is claimed is that on the average the infected

crabs have a more constantly high percentage of fat in the liver than normal males, and this is completely borne out by the quantitative results.

The table shows that, both in respect of glycogen and fat, the infected crabs have a more equable and definite supply of these reserve materials in the liver, that under ordinary conditions of feeding they do not show the same degree of variation and fluctuation in respect to the storage of these substances as the normal males do. Now the normal males are in various conditions according to the period of the life-cycle they happen to have reached—e. g. some are reproducing, others are growing actively and about to moult, etc.—whereas the infected individuals are dominated by one constant condition, the presence of a parasite which is demanding a certain kind of food from its host, principally of a fatty nature. This constant demand leads to a stereo-typed and constant type of metabolism, which is principally characterised by a constantly high elaboration and storage of fat in the liver. It would seem that a normal, though not excessive, supply of glycogen is also present in the livers of infected crabs, but in the case of Carcinus at any rate there is no periodical heaping up of glycogen in the liver or skin for the purpose of a moult, which, in fact, does not occur. From Pott's observations on Pagurids infected with Pelto-gaster it might be surmised that the glycogenic function of the liver is in some way stimulated, leading to frequent moults. Exactly what happens in this case is not really known, but, as has been pointed out, it is at any rate doubtful if the infected Pagurids actually grow any faster than normal individuals, since the infected forms are by no means on the average larger than the normal, which they certainly should be if their frequent moults resulted each time in an increase in size.

We know from other cases that growth and moulting are not always coincident phenomena in Crustacea. Thus, in certain Amphipods the recent work of Mrs. Sexton and Mrs. Matthews (11) has shown that the adult female moults far more

frequently than the male, and yet only attains to about half his size. It is fairly certain that the large quantity of glycogen stored in the liver and skin of Crustacea before the moult is not only used for forming the new skin, but also for the repair and growth of other tissues, so that it is quite possible that the male Amphipod mentioned above really stores more glycogen than the female, although the latter moults more frequently. The anomaly of the hermit crabs moulting without active growth, under the influence of Peltogaster, may therefore be due, not to an increased glycogen storage, but to some other stimulus leading to frequent moults, despite the comparative depression of the glycogenic function. If it could be shown that hermit-crabs parasitised by Peltogaster actually exhibit a comparative poverty of glycogen in liver and skin, despite their frequent moulting, a complete agreement might be shown to exist between this case and that of Carcinus in respect to the glycogenic functions.

In connection with these observations it is of interest to note that in normal Carcinus the adult females never attain to anything like the same size as the adult males, though they presumably moult as frequently. It is highly probable, therefore, that normal adult females, as the result of their breeding activities, lack a plentiful supply of glycogen for the purpose of growth, and if this is the case we are presented with another similarity between the metabolism of normal females and infected individuals, viz. the depression of the glycogenic function.

4. Summary of the Physiological Action of Sacculina on its Host.

The analysis of the morphological changes brought about by Sacculina and Peltogaster on their hosts may be shortly summarised in the statement that these parasites act throughout as feminising agents, converting the male externally and internally, in various degrees, to the female state, and leaving the female either unchanged or else hastening on the adult

female characters, despite the partial destruction of the ovary, which is deprived of its nutrition.

For some time an attempt has been made to find the underlying physiological counterpart of these morphological changes, and it can now be claimed that at any rate in some particulars the feminising influence of the activity of the Sacculina roots has been traced to its physiological cause. We can summarise the physiological action of Sacculina in the statement that the roots of the parasite act the same part in the metabolism of the infected crabs as the ovary of a normal female crab, by taking up from the blood the same fatty material as is required by the ovary, and by stimulating the metabolic organ, viz. the liver, to an increased elaboration of fat. So far we are on certain ground, but in other respects we can trace the feminising action of the parasite, though the interpretation of these results is not so simple. Thus, in the normal female, maturing its ovaries, it has been shown that the blood becomes progressively charged with lutein and fat which are deposited in the ovary, until finally at the shedding of the eggs the blood becomes colourless again. In Sacculinised Carcinus the blood does not become charged with lutein and fat, but the liver is always coloured with the lutein and so are the Sacculina roots, showing that a transference of these materials has occurred, perhaps so rapidly that their presence in the blood cannot be detected. In Sacculinised Inachus the red lipochrome tetronerythrin appears frequently in the blood, but here it is not known whether this pigment is accompanied by fat and lutein which it masks—an occurrence which is often found towards the end of maturity in a normal female.

In regard to moulting in Carcinus, we find that the periodic heaping up of glycogen in the liver and skin preparatory to a moult does not occur in Sacculinised crabs, these individuals never moulting and never growing after once the Sacculina has come to the exterior. We may say, therefore, that there is an inhibition of the glycogenic function both in relation to growth and moulting in infected individuals. It is probable

that in this respect also the infected individuals resemble the
adult normal females, because the latter do not attain to the
same size as the normal males, and this is no doubt due to
their comparative poverty in the reserve substance, glycogen,.
from which growth and the repair of tissue is derived.

Before going on to formulate a theory of the action of the
Sacculina roots in stimulating the fatty and depressing the
glycogenic function of the liver, it is important to realise that
the facts summarised above afford evidence of a physiological
process of considerable general interest. We see that the
Sacculina roots demand and absorb a large quantity of fat
from the crab, and, on the other hand, there is no evidence
of their demanding or taking up glycogen. Now, it might
be supposed that in consequence the crab's liver would be
drained of fat by the parasite, while it would contain an
excess of glycogen which is not abstracted by the parasite;.
yet, as a matter of observed fact, we find the exact
opposite taking place : we find that the extra demand
on the fat made by the parasite is met by an excessive
formation of fat in the liver, while the absence of demand for
glycogen is responded to by a suppression of the glycogenic
function. This is plainly a process of physiological regulation,.
an extra demand being met by an excessive supply.

It is exactly here that we observe an analogy between the
physiological regulation of the metabolism in crabs infected
by Sacculina and the phenomena of regulation met with in
immunity phenomena in general. It is not indeed surprising
that we should meet with such an analogy, because in both
cases we are dealing with the reaction of an organism to a
parasite.

In immunity to bacterial diseases, whatever may be the
nature or place of origin of the immune substances, it is at
any rate clear that we are presented with the formation in
excess of substances which are being linked on to and fixed
by the parasite. Whether these substances originate from
the tissues attacked by the parasite, or from the phagocytes.
which attack the parasite, at any rate an over-production of

them occurs which results in the flooding of the blood by the immune substances, which are therefore present at a future time, either to block the access of the parasite to the tissues, or else to fix on to the parasite and disable it. It is the undoubted fact of this production in excess of a substance which is in some manner being linked on to the intruding parasite which confers on Ehrlich's side-chain theory of immunity its ready acceptation in principle, however much authorities may differ as to its application and detailed working out. But it is exactly in the root principle of Ehrlich's side-chain theory, viz. in the principle of the regeneration in excess of a substance which is being linked on to a parasite, that we find an extraordinarily close parallel in the reaction of the crabs to Sacculina, and we therefore may feel justified in casting our theory of this reaction into terms of the side-chain theory.

If we were to frame this theory in its simplest possible form, we might say that the reaction is brought about by the Sacculina roots seizing on the fatty side-chains of the liver, which, in consequence, are regenerated in excess. We know, however, that this simple statement does not cover two essential facts : first, that the exchange takes place through the medium of the blood, and second, that fat is not present as such in the blood, but in some soluble form in combination with some other material. We must, therefore, represent the Sacculina roots as in some way seizing on the fatty part of this combination in the blood, and thus setting free the other part to take up more fat from the liver and convey it again to the Sacculina roots.

An illustration of the method by which such a reaction might be conceived to take place is given in the subjoined diagram.

We may suppose that the proteid molecules of the blood (P_1 and P_2) are provided with side-chains (L, L) which act as fat-links, having the power of seizing on and combining with fat-molecules. P_2 in the diagram is represented in the act of seizing on such fat-molecules in the liver on the right-hand

side of the diagram. When the combination between fat-link and fat has taken place, the proteid molecule is detached
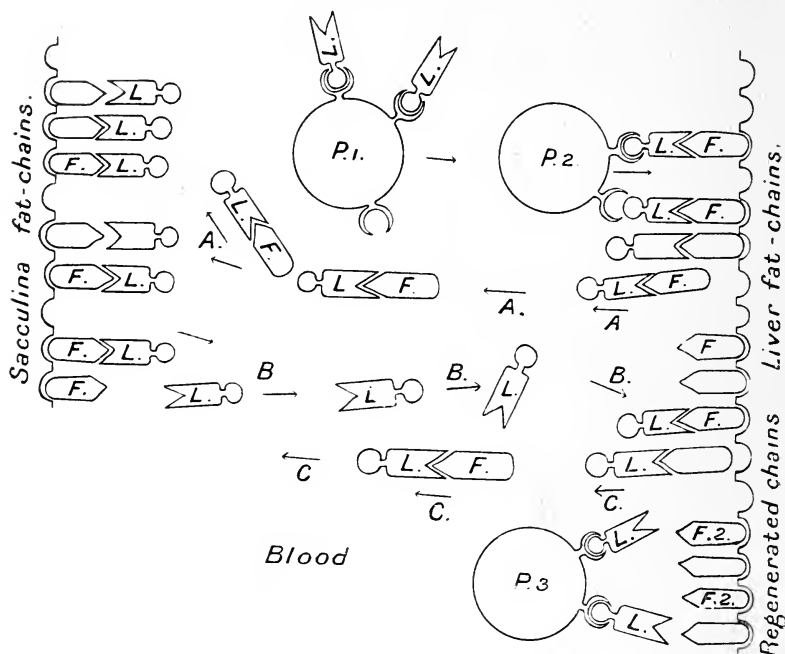
EXPLANATION OF DIAGRAM.

The Sacculina fat-chains are represented by the linked half-circles on the left, those of the liver on the right. Into them are fitted the fat-molecules (F.). Floating free in the blood a proteid molecule (P_1) is represented which carries two fat-link side-chains L. In P_2 two of the fat-links have seized on fat-molecules in the liver and are detaching them. The passage of fat and fat-link combinations, F L, from liver to Sacculina is indicated by the arrows A, A, A. The fat-molecules are taken up by the Sacculina side-chains, and the free fat-links are liberated into the blood, L. They pass back to the liver as indicated by the arrows B, B, B, and fix onto new fat-molecules in the liver, which they detach and carry again to the Sacculina (arrows C, C, C). This continued process leads to regeneration of new fat-chains with fat in the liver, indicated at F_2, which will be attacked by fresh fat-links, derived either from the above process or from new proteid molecules, P_3.

from its fat-link, and the combined fat-link + fatty molecule is broken away from the liver and floats freely in the blood. We suppose that this process occurs in the normal metabolism of the animal.

Now, when the Sacculina roots begin their activity they seize on the fatty molecules in the fat-link + fat combinations which are free in the blood, as shown on the left-hand side of the diagram, and in so doing they liberate large numbers of free fat-links. These free fat-links, with an unsatisfied affinity for fat, travel again to the liver, where they fix themselves onto fresh fat-molecules in the liver, as shown in the middle part of the right-hand side of the diagram.

Now, this process of constantly fixing on the fat-molecules of the liver leads to a regeneration in excess of the fat side-chains in the liver, and as more and more fat-links from the proteid molecules attach themselves to these regenerated fat side-chains, the process goes on in an ever-increasing ratio.

The result of the process will clearly be to flood the blood with a large number of fat-link + fat combinations and of free fat-links, so that the total composition of the blood will be materially affected. It may be urged in criticism of this theory that it is fanciful and artificial, but though we are far from claiming that the representation of the facts by our symbols approaches the chemical reality, yet we have actual evidence in fact for each step of the process.

We may, of course, replace the Sacculina roots on the left-hand side of the diagram with the ovary of a normal female crab, and in this case we have actual evidence of the alteration of the composition of blood during the growth of the ovary in the flooding of the blood by lutein and fat, which has been shown to occur.

Now, we know from a variety of evidence that the development of certain secondary sexual characters depends for its stimulus on substances carried about the body in the medium of the blood or body-fluids. If, then, our conception of the action of the Sacculina roots or ovary is correct, we have shown how they can produce substances in the blood and

alter the composition of this medium, and thus lead to the stimulation of the development of the female secondary sexual characters by means of the excess of fat-links and fat-link + fat combinations.

It is also not difficult to explain on this theory why it it happens that the infected male crabs, on recovery, may regenerate an ovary instead of a testis, because the fat-links and fat which are present in the blood are the specific food-material of the ovary, and hence the indifferent germ-cells which remain at the end of infection are supplied with the specific female food-material and naturally grow into ova. In fact, by showing that the substances which stimulate the development of the secondary sexual characters are identical with the specific food-materials or food-carriers of the repro-ductive gland, we not only gain a rational explanation of the effect of Sacculina on its hosts, but we can put our finger on the common formative substances which lie at the back of sexual differentiation, both primary and secondary.

In the special form in which our hypothesis was presented we supposed that the result of the Sacculina's or ovary's activity was to load the blood with two principal substances, the free fat-links and the fat-link + fat combinations. Either or both of these substances may be concerned in stimulating the development of the secondary sexual charac-ters. There may even be a further series of complicated reactions which are initiated by the presence of the unsatisfied fat-links in large numbers. To determine which of these alternatives is correct must be the task of the future, and it is not unlikely that we may find a means of testing and extending the hypothesis. The criticism has been urged against statements of this hypothesis which I have previously put forward, that the mere presence of fat in the blood could not be the direct cause of the development of the secondary sexual characters. It will be recognised from the fuller explanation of the mode of action of the Sacculina roots which has now been given, that the mere presence of fat in the blood is not claimed as the cause, but as an accompani-

ment and sign of more deep-seated changes, which may involve perhaps several kinds of side-chains attached to proteid-molecules or cast loose in the blood.

Another question which must be left to the future to decide is, what may be the corresponding action exerted by the testis of the male upon the metabolism and on the composition of the blood, what food-particles is it seizing on, and of what side-chains or linkages is it stimulating the formation? For answering these questions we have at present little or no data.

We are now in a position to explain how the theory put forward above differs from and is superior to the Hormone theory of sexual development as held by the great majority of physiologists. It is clear that both theories may be legitimately described as "internal secretion" theories as long as we leave the mode of production and of action of the internal secretion entirely vague, but if we pay attention to those two not unimportant considerations, we find that the account given of them by the two theories is entirely different, and I submit that the account given by the Hormone theory is erroneous and not supported in fact.

According to the Hormone theory, the Gonad produces an internal secretion or hormone which it pours into the blood, and which stimulates the appropriate secondary sexual characters to develop. The method by which its adherents attempt to prove this theory is by injecting extracts of the Gonad which contains this substance in the hope that the development of the appropriate secondary sexual characters will be called forth. Partly by judging from the analogy of other cases, and partly by trusting the specious results of experiments designed to prove the case, it is not too much to say that the majority of physiologists and zoologists believe that the Hormone theory is experimentally proved for the reproductive organs. Whilst admitting that these experiments should receive careful consideration and repetition—especially the latter—I cannot agree that they are in any case conclusive, while sources of error exist as far-reaching and as difficult of

detection as those which can be shown (**10**) to invalidate the apparently well-established results on the thumb of the frog.

It is clear that if the theory developed in this paper is well founded, we should not expect that injections of ovarian or testicular substances, or of substances derived from the Sacculina roots, would have any effect in calling forth the development of sexual characters. There is no reason for supposing that an emulsion or extract of these organs would contain the fatty side-chains in a condition capable of assimilation, and of setting on foot the complicated nexus of metabolic processes which results in the progressive alteration of the liver and of the blood.

We have seen that the whole effect of Sacculina on its hosts is consistently explained by the theory we have adopted, a theory which may be described as metabolic stimulation.

Let us see how far short the Hormone theory falls in a similar attempt. Since the infected males develop female secondary sexual characters in the absence of an ovary, the Hormone theory offers us two alternatives : either the Sacculina roots secrete a hormone which acts on the crab, or else the mere suppression of the testis liberates a hormone from the crab which brings about the secondary sexual changes.

To take the last alternative first, it is quite possible that the mere suppression of the testis might call forth the development of female secondary characters, but it is difficult to see how the mere suppression of the testis should make the crab subsequently develop an ovary. But even if we grant this highly improbable result, why should the suppression of the ovary in the young female crab influence the latter to assume prematurely adult female secondary characters ? The explanation in fact falls to pieces when we try to apply it to the whole of the phenomena.

It is the same with the other alternative, viz. that the Sacculina roots secrete the hormone. This would explain why the female secondary sexual characters should be developed, but how could it explain the subsequent forma-

tion of ova in the regenerated testis of infected males? It is no part of the Hormone theory that the hormone which the reproductive organ produces is itself the condition of formation of that reproductive organ, but it is an integral part of our theory that the substances produced in the blood for the nutrition and for purveying the nutrition of the Sacculina roots (or ovary of the crab), act as one and the same stimulus for the development of the secondary sexual characters and for the growth of the Sacculina roots (or ovary). The probing of the facts of parasitic castration, therefore, may lead us to the conviction that the Hormone theory in its generally accepted form, whatever may be its fate in other branches of inquiry, is destined only to play upon the shallows and not to illuminate the depths of the physiology of reproduction.

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Studies on Parasitic Protozoa.

I. The Flagellate Polymastix and its Affinities with the Trichonymphida.

By

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With Plate 18 and 1 Text-figure.

INTRODUCTION.

THE Protozoa known as Trichonymphidæ, parasites of the gut of certain orthopterous insects, have for long presented difficulties to the systematist.

The early observers of the Trichonymphids, Leidy and Grassi, worked chiefly on the more highly specialised genera, such as Pyrsonympha and Dinennympha, and not unnaturally they placed these forms among the Ciliata, to which they bear a strong superficial resemblance. Other authors, dissenting from this view, preferred to regard the Gregarinida as the nearest allies of this perplexing group.

Bütschli's observations on Lophomonas (1878), from the cockroach, put a different aspect on the case. This form, in most respects simpler, and probably more primitive, than the trichonymphids from termites, had obvious affinities with the Flagellata. Kent (1880) formed for it a special family, the Lophomonadidæ, which he placed in the neighbourhood of such flagellates as Trichomonas, Tetramitus, and Hexamitus. Grassi discovered Joenia annectens (1885) which is a link between Lophomonas and the more

elaborate forms, and strengthened the view that the tricho-nymphids had sprung from a flagellate stock, a view that the recent work of Janicki (1910) and others has still further confirmed.

The object of the present paper is to point out that, while the affinities of Lophomonas are with the POLYMASTIGINA, of Doflein, as Kent long ago suggested, yet, within that order, it is to the hitherto little-known genus Polymastix that we must look for further data of phylogenetic interest. (See Mackinnon, 1912).

POLYMASTIX, BÜTSCHLI.

Habitat and Previous Records.

Polymastix is a small flagellate which has been found parasitic in the alimentary canal of certain larval insects, such as Melolontha, Cetonia, Orycytes,[1] and Tipula.

It was first described by Grassi (1882), as Trichomonas melolonthæ. Bütschli (1884) removed it from the genus Trichomonas, and formed for it a new genus Polymastix, characterised by four equal, forwardly directed flagella, and a firm striated periplast. Künstler's account (1882) brought in some confusion by his stating that there might be as many as six flagella.[2] This author also suggested that the striations on the periplast might be adherent bacteria.

Polymastix was not further investigated until 1911, when Hamburger briefly described the flagellate as it is found in Melolontha and Cetonia. Contemporaneously with Fräulein Hamburger, I had been working on Poly-mastix from the hind-gut of the larva of Tipula, sp. I published a short account in a preliminary note in 'Parasi-

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[1] I am indebted to Dr. Carlos França for this record of another coleopterous host of Polymastix.

[2] This statement led Alexeieff (1911) to place in the genus Poly-mastix as P. batrachorum, a flagellate with six flagella from Triton tæniatus, for which he has since (1912) been obliged to erect a new genus, Hexamastix.

tology' (1912), an account which I am now able to amplify
in certain particulars that seem to me to bear on the probable
affinities of Polymastix with Lophomonas.
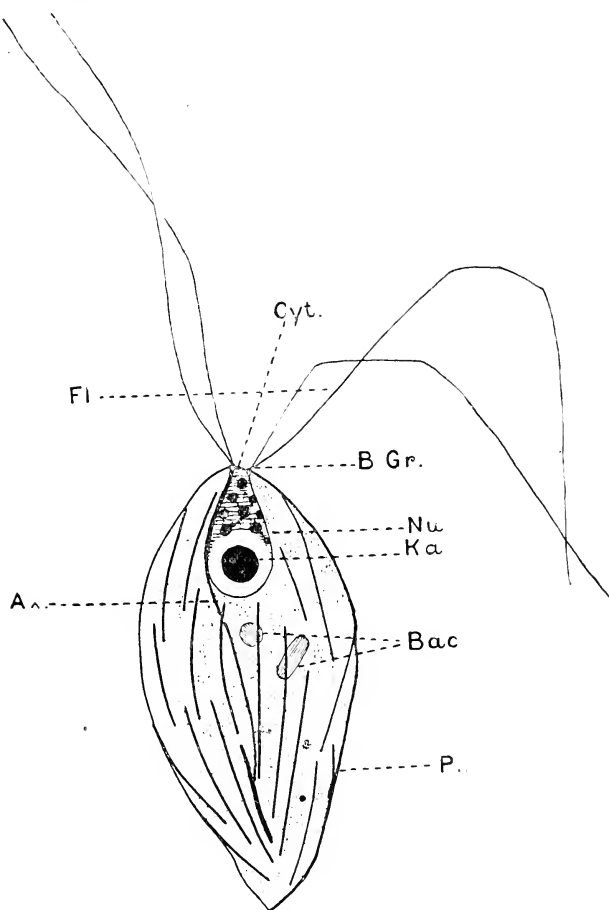
Polymastix from Tipula. Characteristic flagellate individual,
   showing four flagella, ribbed periplast, pear-shaped nucleus,
   with karyosome, cytostome, basal granules, axostyle, and
   ingested bacteria.

Description.

Body pear-shaped to spindle-shaped; the anterior
end rounded, the posterior end tapering, or often

forked and otherwise "deformed." Four flagella[1] arise from two basal granules at the extreme anterior end in two groups of two; they are sub-equal in length,[2] and are considerably longer than the body; there is no "Schlepp-geissel." A cytostome lies between the basal granules. Periplast relatively thick and rigid, raised into numerous folds or ribs, which run in a direction approximately parallel to the long axis. Cytoplasm finely alveolar, containing numerous ingested bacteria. Axostyle usually present, but feebly developed. Nucleus immediately behind the basal granules; sometimes spherical, more often pear-shaped, with large karyosome surrounded by a clear zone, which is surmounted by a group of chromatin granules. Extra-nuclear granules of chromatin (?) may also occur.

Dimensions, $7\,\mu$ to $15\,\mu \times 4\,\mu$ to $6 \cdot 5\,\mu$.

Whether the Polymastix found in Tipula is to be considered as a species distinct from the type-species, Polymastix melolonthæ (Grassi), it is difficult to say. Hamburger considers that the same species lives both in Melolontha and in Cetonia. I have recently had the opportunity, through the courtesy of Dr. Carlos França, of examining a preparation of Polymastix from the gut of a larva of Oryctes nasicornis, and it appeared to me indistinguishable from the flagellate of Tipula. The evidence, then, is in favour of the view that a number of insects of similar feeding habits may be parasitised by Polymastix melolonthæ (Grassi).

The following observations have reference to the parasite as found in Tipula.

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[1] It is to be noted that certain undoubted Trichonymphids, such as Devescovina striata, have only four flagella.

[2] Authors—Hamburger, Jollos, Alexeieff—state that the flagella are unequal in length. I think that this is so only in individuals that have recently undergone division; in such a case two flagella may be regrown, and for some time these will appear shorter than the rest (vide infra).

The Pellicula.

One of the most characteristic features of Polymastix is the thickened periplast or pellicula, which gives the body a certain rigidity and stiffness, and prevents any " metabolic " altering of shape.   Sometimes the empty periplast is found persisting after the disappearance of its softer contents. A distinct gap in its continuity may be observed at the extreme anterior end ; this gap marks the position of the cytostome.   The surface of the periplast is raised up into a number of thickened ribs or ridges ("côtes" of French authors), which stain deeply with iron-hæmatoxylin.   These are not continuous from end to end of the body, but, while approximately parallel to the long axis, they fall into little groups of two or three, often inclined at considerable angles. Grassi suggested that these structures, which appear as dark striations in stained preparations, might be trichocysts; Künstler held them for adherent bacteria.   Sometimes the periplast may be seen fraying off, so that the " ribs " project from the surface, when they look very like adherent bacilli (Pl. 18, figs. 12 and 14).

Hamburger compares these striations with thickenings in the periplast of Euglena.

Now, it is of interest to find that such pellicular thickenings are eminently characteristic of the Trichonymphid.   " Für die meisten Gattungen ist das Auftreten von Stäbchenför-migen Bildüngen in der Pellicula charakteristisch" (Doflein, 1911).   Lophomonas striata, Bütschli, in particular, is provided with a thick, striated periplast, and Janicki's excellent account and figures (1910) leave no doubt that this structure presents the same general characters as in Polymastix.[1]

[1] Alexeieff shares my opinion as to the affinities between Poly-mastix and the Lophomonadidæ—" Le genre Polymastix pourra être placé dans la famille des Lophomonadidæ, ou tout au moins con-sidéré comme une forme de transition entre les Polymastigines et les Lophomonadidæ (Trichonymphines) (1912)."

The Nucleus.

The nucleus presents very different appearances according to the intensity of the staining. In badly differentiated individuals stained with iron-hæmatoxylin it has the aspect figured and described by Hamburger, i. e. that of a pear-shaped body at the anterior end of the organism, containing a voluminous karyosome. Better staining reveals a clear zone surrounding the karyosome, and sometimes containing a few grains of chromatin. Above this is a dark-staining cone-shaped area, in which lie a number of chromatin granules supported in a delicate reticulum. These structures are enclosed within a definite pear-shaped membrane, the upper borders of which touch the basal granules. This is the most usual aspect. Sometimes, however, the nucleus wanders from its anterior attachment, and appears as a sphere containing a karyosome, and surmounted by a group of extra-nuclear granules ; in this condition it is strikingly like the nucleus of the Monocercomonas that occurs abundantly alongside Polymastix.

The pear-shaped nuclear apparatus presents a certain resemblance to the "calyx" and contained nucleus of Lophomonas. Certain of Janicki's figures of Lophomonas blattarum, Stein, are very suggestive in this connection— I refer more particularly to figs. 2B, D and E on pl. vi of "Untersuchungen an Parasitischen Flagellaten," I ('Zeit-schrift f. Wissenschaft Zool.,' 1910). There, within a cup-shaped area, limited by a membrane, lies a spherical nucleus, which contains an eccentric karyosome surrounded by a clear zone and surmounted by a dark-staining mass containing scattered chromatin granules. In Polymastix there is no trace of the "collar" and other circumnuclear structures, though the cytostome may bring in another complication.

The Axostyle.

My preparations of Polymastix show the presence of a feebly developed axostyle, which had been overlooked by

previous observers. The axostyle arises from the neighbour-
hood of the basal granules, passes down one side of the
nucleus and pursues a curved course for some distance below;
it seldom extends into the posterior third of the body, and is
often completely absent.

The feeble development of the axostyle is no doubt asso-
ciated with the presence of the rigid pellicula, which makes
internal skeletal support superfluous (cf. the different degree
of development of the axostyle in the plastic Lophomonas
blattarum and the rigid L. striata).

The relations of the axostyle to the nuclear apparatus seem
to be different in Lophomonas. There Janicki describes
the central fibrils of the axostyle as traversing the calyx and
nucleus and ending beneath the circle of basal granules:
"Irgend welche direkten Beziehungen zwischen Basal-
körnern und Kern konnten in Lophomonas nicht beo-
bachtet werden." Furthermore, the axostyle of Lopho-
monas is re-formed by the division spindle, as in Tricho-
monas batrachorum, Perty, and other allied flagellates.
In Polymastix I could find no sign of this.

Division.

Early observers of Polymastix noted something unusual
in the mode of division. "Wahrscheinlich erfolgt die Ver-
mehrung durch Querteilung" (Doflein, 1911). Hamburger
failed to find any evidence on this point.

My own experience has been that, though the flagellate may
be present in enormous numbers, it is very hard to find indi-
viduals in division. Consequently I am able to supply only
the outline of the process, but I have been so much struck by
the general similarity between this and the division in Lopho-
monas that I feel justified in publishing the figures.

It should perhaps be noted first that the individuals with
forked posterior ends, so commonly met with, are not dividing
forms. I have watched the living organisms for long periods
to satisfy myself of this, nor is there any indication of nuclear

division in such forked forms as I have found stained (Pl.
18, fig. 11).

What happens in division is as follows. The spherical
karyosome elongates, becomes dumbbell-shaped (Pl. 18, fig.
1), and finally divides into two (Pl. 18, fig. 2). Each
daughter-karyosome is surrounded by a clear zone, and takes
over with it a certain number of the supra-nuclear chromatin
granules (Pl. 18, fig. 3); the reticulum supporting these
chromatin grains has previously become much fainter, and
they show a tendency to wander beyond the limits of the
nuclear membrane.

One of the daughter-nuclei so formed remains in the original
position at the extreme anterior end of the body; the other
wanders back just below the periplast until it comes to lie at
the posterior end (Pl. 18, figs. 4 and 5). Meanwhile the
body has shown signs of elongation, a constriction appears
halfway, and the two halves, after hanging together for some
time, gradually separate (Pl. 18, figs. 6 and 7). I have not
been able to find the last stage of separation in the stained
material, but I saw it once in the living organism.

The fate of the flagella and the basal granules is rather
curious. Generally one basal granule and two flagella go
over with the travelling nucleus; sometimes a fine fibril is
seen to extend from the basal granule to the nucleus (Pl.
18, fig. 4). In such a case each daughter-nucleus must
re-grow two flagella; in this way I explain the occurrence of
individuals with exceedingly unequal flagella. The sprouting
of the new flagella is preceded by division of the basal granule
into two (Pl. 18, figs. 4 and 5). But sometimes all four
flagella remain behind with the stationary nucleus (Pl. 18,
figs. 8 and 9), while the wandering nucleus moves off with-
out any, and presumably must re-grow all four when it reaches
its destination. If the flagella grow out from the basal granules,
as seems most probable, we should expect that, in this case,
the wandering nucleus would receive its share of these, but I
have not been able to observe this.

The axostyle may persist for some way through the division

process (Pl. 18, fig. 8); then it seems to be absorbed, and I have seen no indication as to the way in which it may be re-grown.

Now, while the process described above is much simpler than what takes place in Lophomonas, there are some striking resemblances, which a comparison of my figures with those of Janicki will at once reveal. This is particularly well seen in the case of Lophomonas striata, where the daughter-nuclei, separating from a division-spindle which is parallel with the long axis, migrate, the one to a position alongside that occupied by the mother-nucleus, the other to the posterior end of the body. The flagella are then re-grown, a constriction appears in the middle of the body, and the two halves separate. As Janicki shrewdly points out, " Es konnte ja diese Art der Körperteilung auf den ersten Blick als eine Querteilung gedeutet werden; das ist sicher nicht der Fall, sondern die wachsenden Plasmamassen, anstatt durch die Längsteilungebene einfach nach rechts und links ausein-andergeklappt zu werden, gleiten sozusagen an derselben polarwärts, bis sie von einander lostrennen" (Janicki, 1910, p. 306).

The wandering of the nucleus of Polymastix also recalls very vividly the description of the division of Lophomonas blattarum: " Die zweikernigen Formen sind äusserst lebhafte Tierchen. . . . Die Korperpartien welche den Kern mit den zugehörigen Nebenorganellen beher-bergen sowie die Flagellenschöpfe, sind befähigt, über den ubrigen Körperplasma in oberflächlicher Schicht mit grosser Geschwindigkeit hin und her zu gleiten, so dass sie bald nahe aneinander liegen, bald nach den entgegengesetzen Polen sich entfernen" (Janicki, 1910, p. 273).

Encystment.

As to the mode of encystment in Polymastix I can say very little. In preparation the organism loses its flagella and the nucleus migrates to the centre of the body (Pl. 18, figs. 12, 15 and 16). Meanwhile the periplast shows signs of

disintegrating, and the "ribs" fray off, exactly as in Lophomonas striata. I have seen no completely formed cysts nor any trace of division in the encysted state.

SUMMARY.

Whatever be the relationship of the Lophomonadidæ with other Trichonymphids, recent research has clearly indicated that their affinities on the flagellate side are with the order POLYMASTIGINA, Doflein, and in this order with the family Polymastigidæ, Bütschli.

The genus Polymastix, Bütschli, itself shows certain structural features that greatly strengthen this view. While the comparison must not be pushed into too great detail, the evidence so far collected seems strong enough to make mere coincidence of resemblance improbable. The points of similarity may be summed up as follows :

(1) The similar character of the ribbed periplast in Lophomonas striata and in Polymastix.

(2) Certain points of resemblance in the nuclei of the two.

(3) The peculiar nature of the division process, " apparently transverse," in Lophomonas striata and in Polymastix.

To this may perhaps be added the fact that Lophomonas and Polymastix, as far as the records show, are both parasites of insects only.

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EXPLANATION OF PLATE 18,

Illustrating Miss Doris L. Mackinnon's paper, "Studies on
Parasitic Protozoa.—I. The Flagellate Polymastix
and its Affinities with the Trichonymphida."

[All figures drawn to scale (× 4000 approx.) under Zeiss comp. oc. 12
and 2 mm. apochromat. The stain employed was in all cases Heiden-
hain's iron-hæmatoxylin after fixation with sublimate-alcohol.]

Fig. 1.—Polymastix from Tipula, showing dividing karyosome.

Fig. 2.—A later stage in the division process: the daughter-karyo-
somes have separated.

Fig. 3.—Separation of the nuclei.

Fig. 4.—One nucleus remains at anterior end; the other migrates.
Note that the basal granules have divided, and one of the missing
flagella has been re-grown in each case.

Fig. 5.—A slightly later stage. The body of the flagella shows signs
of elongating.

Figs. 6 and 7.—The wandering nucleus has reached the posterior end
of the body. In fig. 7 the elongated body is beginning to constrict in
the middle.

Fig. 8.—The migrating nucleus has moved off without being accom-
panied by flagella. Note persistent axostyle.

[1] Fuller references to the literature on Trichonymphid may be got
by consulting the bibliography at the end of this work.

Fig. 9.—A later stage of fig. 8. The migrating nucleus approaches the posterior end of the body.

Fig. 10.—" Tailed " individual of Polymastix.

Fig. 11.—" Forked " individual.

Fig. 12.—Preparation for encystment. Periplast fraying off, flagella disappearing. Nucleus has taken up a central position.

Fig. 13.—" Deformed " individual with displaced nucleus. Possibly an early stage of encystment.

Fig. 14.—" Ribs " of the periplast fraying off. In this condition they look very like adherent bacteria.

Fig. 15.—Encysting Polymastix. The flagella have disappeared, and the nucleus has moved to the centre of the body.

Fig. 16.—Encysting Polymastix. A rather later stage. The ribs of the periplast have almost all disappeared. Note the basal granules, surrounded by a clear zone.

Studies on the Development of the Veno-Lymphatics in the Tail-region of Polistotrema (Bdellostoma) stouti. First Communication: Formation of the Caudal Hearts.

By

William F. Allen.

With Plates 19-21.

I. Introduction.

The purport of this and a following communication is to record certain facts and conclusions concerning the origin of the veno-lymphatics in the tail region of .Polistotrema (Bdellostoma) stouti. This paper will include a description of the adult condition of these vessels and the manner in which the caudal hearts are formed. Shortly I hope to follow with a second communication on the development of the veno-lymphatics in the tail region.

For several years I have been gathering together a series of Polistotrema embryos from Monterey Bay, California. Miss Julia B. Platt of Pacific Grove, California, Prof. G. C. Price of Stanford University, and Miss Julia Worthington of Cincinnati, Ohio, have generously added to my collection. All of the embryos were obtained by one Chinese fisherman, and unless someone was present to take charge of the material he was likely to preserve partly decayed embryos. Consequently a considerable portion of my material had to be discarded after sectioning; especially was this true of stages

between 15 and 20 mm. From 20 to 30 mm. and from 48 to 85 mm. my series are good and fairly complete.

My own specimens were all fixed in Tellyesnicky's potassium-bichromate-acetic mixture, embedded in paraffin, cut transversely 10 microns thick, and stained in either Heidenhain's iron or Hansen's hæmatoxylin and counter-stained with a saturated alcoholic solution of orange G plus a little acid fuchsin. From my experience with the injection method I am satisfied that in many cases it would have only demonstrated the later stages of development, namely, the stage where the mesenchymal spaces had united to form a continuous canal, unless perchance there had been an extravasation of the injection mass, breaking down the mesenchyme separating these cavities, and possibly going still further. Upon injecting the lymphatics of fishes I have frequently broken down the delicate walls separating the lymphatics from a vein, filling the veins just as one sometimes fills the veins in the liver upon injecting the bile-vessels. In well-preserved material there is no reason whatever for regarding the mesenchymal spaces found in the region of growing lymphatics as artifacts, for if such, they would occur elsewhere in the mesenchyme.

My previous studies on the lymphatics of fishes have indicated that this system is more closely allied to the venous system than is the case in the higher vertebrates. This is especially true of Polistotrema, where these vessels always contained blood, although it should be stated that no direct connections were found with the arterial system. The primitive vertebrate doubtless had no distinct lymphatics other than the veins, which would also function for lymphatics, a portion of which in their later phylogeny may have differentiated into lymphatics. If this be true, it is reasonable to suppose that the lymphatics have the same origin, ontogenetically, as their more primitive ancestoral veins, of which considerable data shows that the larger veins do not arise as sprouts from a central venous centre, but rather grow from periphery to the centre, very much after the manner of the formation of the blood islands, mesenchymal spaces, in the

blastoderm, to form the omphalo-mesenteric vessels, and their endothelial walls are formed by a flattening of certain of the mesenchymal cells; while in a somewhat similar manner some of the enclosed mesenchymal cells are transformed by enlargement and rounding into red corpuscles. Prof. Scammon, in his recent work on Squalus, clearly shows from careful reconstructions that the anterior cardinals in this shark are laid down as isolated vessels before any communication is established with the venous part of the heart, and that this connection does not occur until a much later stage. In an early stage he finds a rather strong cephalic arterial connection, which he informs me likely gives rise to the anterior portion of this vein, but he is of the opinion that the posterior portion has an independent origin (probably from the union of certain mesenchymal spaces).

On account of the resemblance of the lymphatics to venous sinuses in Polistotrema I shall describe them as veno-lymphatics, which is indicative of a more generalized type, approaching the more primitive venous stage. It will be apparent that this use of the term differs from that made when it is applied to lymphatics arising ontogenetically from veins.

Notwithstanding the fact that the caudal heart and some of the caudal vessels of Myxine[1] and Polistotrema have been accurately described in the adult by Retzius, Klinckowström, Greene, and Favaro, it has seemed advisable for the sake of comparison to include here a brief description of these vessels as they appear in the adult from dissection and from serial transverse sections of a 20 cm. adult and an 85 mm. embryo that had assumed practically adult conditions.

II. Veno-Lymphatics in the Adult Polistotrema.

Caudal Hearts (Figs. 1 and 4, *R.* and *L. Cau. H.*).—Like Anguilla (eel), the caudal hearts are paired pulsating hearts

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[1] A complete bibliography will be furnished in the next communication.

of considerable size, situated side by side, near the tip of the tail. They are nothing more than pronounced swellings of the anterior portion of the right and left forks of the caudal veins, which completely cover the lateral surfaces of the anterior third of the median ventral cartilaginous bar. At the point where the caudal veins (anterior portions of the caudal hearts) unite to form the common caudal vein their walls extend into the posterior end of the vein as valves, thus preventing a back flow of blood or lymph into the caudal hearts.

In the 20 cm. series the greatest caudo-cephalic diameter of the caudal heart is 2·52 mm., its greatest dorso-ventral diameter is 1·52 mm., and its greatest median-lateral diameter is ·1 mm. The corresponding measurements in the 85 mm. series are 1·1 mm. by ·47 mm. by ·1 mm. These measurements would indicate that the heart is about twice as long as it is high.

Both hearts in section (Fig. 1) have the general form of pyramids with their apices pointing dorsad. Usually both are filled with red corpuscles, but ordinarily one is distended more than the other. If Greene's observations are correct, that they contract alternately, it would account for there being more blood in one than in the other.

The inner wall of the heart is endothelium, surrounded by a layer of dense white fibrous tissue, which binds it inward to the median ventral cartilaginous bar and laterad to the musculi cordis caudalis. According to Favaro this layer abounds in elastic fibres, but none were visible in my sections, although it should be stated that I employed no specific stains. The musculi cordis caudalis (Fig. 1, M. C. C.), which functions as a partial myocardium, is attached anteriorly to the lateral process of the median ventral bar, and its striated longitudinal fibres, after passing posteriorly over the lateral surface of the caudal heart, become attached to the median ventral bar, and to a slight extent to the bases of some of the ventral or anal fin radials. This muscle later on will be shown to be more intimately related to the

myotomes of the great lateral or body-wall muscle than it is to the heart.

Concerning the innervation of the musculi cordis caudalis, in the 20 cm. and 85 mm. series there are four ventral spinal nerve rami that travel caudo-ventrad between the myotomes and the m. cordis caudalis and could innervate the m. cordis caudalis, but only from the third (counting from cephalo-caudad) were any fibres seen going to this muscle. The first of these nerves on the left side had its motor nucleus and its spinal ganglion considerably cephalad of the m. cordis caudalis or the caudal heart and slightly in advance of the forking of the caudal artery and vein. It travelled gradually caudo-ventrad, and in the region of the anterior end of the heart it passed between the myotomes and the m. cordis caudalis, slightly nearer the former, which it innervated; but absolutely no fibres were seen going to the m. cordis caudalis. The motor nucleus and spinal ganglion of the second nerve was situated immediately dorsad of the passing of the first ventral ramus between the myotomes and the m. cordis caudalis. Like the first nerve it travelled caudo-ventrad between the myotomes and the m. cordis caudalis in the region of the anterior end of the caudal heart, but did not innervate the m. cordis caudalis. The nucleus or the third motor root and the spinal ganglion was dorsad of the crossing of the second ventral ramus between the myotomes and the m. cordis caudalis, and at the posterior end of the heart the ventral ramus of the third spinal nerve passed between the myotomes and the m. cordis caudalis, innervating both. In the case of the 85 mm. embryo the left caudal heart was in an embryonic condition at the point of innervation of the m. cordis caudalis by the ventral ramus of the third spinal nerve, consisting only of the original left caudal vein and one mesenchymal cavity some distance dorsad of it. The nucleus of the fourth motor root and ganglion was situated immediately dorsad of the innervation of the m. cordis caudalis, and, like the other ventral rami, the fourth had a caudal ventral course between the myotomes and the extreme caudal end of

the m. cordis caudalis, which is some little distance behind the caudal heart, but, unlike the third ventral ramus, it innervated only the myotomes.

The four ventral spinal nerve rami described above in relation to the m. cordis caudalis are of considerable value as landmarks for studying the limits of the embryonic caudal hearts or in the adult they demonstrate that the m. cordis caudalis extends over three segments, while the caudal heart is somewhat shorter.

The peculiar state of the spinal cord in the region of the spinal ganglion, resembling lateral ventricles of the central canal, and an expansion of the central canal dorsad and laterad in the form of rhombic lips, is reserved for a later paper.

Most remarkable is the extremely rich blood supply in the m. cordis caudalis of the 20 cm. series. Vessels of considerable size traverse long distances in this muscle, but their connections outside the muscle are difficult to make out. It can, however, be reduced to one artery and three veins if the caudal heart is left out. The first or most anterior vessel noticed in this region on the left side is an inter-segmental vein, consisting only of a ventral or hæmal branch which passes between the anterior portion of the m. cordis caudalis and the myotomes, dorso-cephalad, to terminate in the caudal vein a little in advance of the point of its bifurcation, and so far as could be determined it received no branches from the m. cordis caudalis. The second vessel is also a vein, but it drains several segments and terminates in the left caudal heart a little anterior of its centre. To trace backward, it pierces the m. cordis caudalis and then continues gradually caudo-ventrad between the m. cordis caudalis and the myotomes to the posterior end of the heart, and it is this vein that apparently collects the blood from the m. cordis caudalis. A little anterior of the middle of the heart a typical inter-segmental artery is given off from the lateral side of the left caudal artery. It soon separates into dorsal and ventral branches, and the latter travels caudo-ventrad with the second

ventral spinal nerve ramus, between the m. cordis caudalis
and the myotomes, probably supplying both, though I could
trace no branches going to the m. cordis caudalis. The
fourth and last blood-vessel visible in this region is a vein,
which terminates in the extreme dorso-lateral side of the left
caudal heart, and to trace backward it passes over the m.
cordis caudalis to the left caudal artery, which it follows for
a short distance, and then divides into a large dorsal branch
and a short ventral branch; the latter drains only the
myotomes.

There is doubtless considerable variation concerning the
arrangement of these inter-segmental vessels in different
individuals, as will be shown in comparing the 20 cm. to an
85 mm. series, and also on the opposite sides of the same
individual. In the 85 mm. embryo only three intersegmental
vessels were noted. The first two were normal inter-
segmental arteries and the third was a vein. The vein in
many respects is homologous to the second vein of the 20 cm.
specimen; its point of termination in the heart is not only
much lower, but it is also much further caudad, being where
the heart is still in embryonic condition.

According to Greene the caudal heart acts on the principle
of a double force pump, the two m. cordis caudalis contracting
alternately, and their rhythmic contractions are said to be
governed by an automatic heart centre situated in the posterior
end of the spinal cord.

Ventral Veno-lymphatic Trunk (Figs. 1, 2, 4 and 4 A,
V. V. or R. and L. V. V.).—For convenience, and in part on
embryological grounds, this important canal, which collects
the lymph and blood from the entire tail region to discharge
it into the caudal hearts, can be divided into two portions.
First, an anterior portion (Figs. 1, 2 and 4, V. V. or R. and
L. V. V.), which travels along the base of the anal fin imme-
diately below the mucous sacs. Sometimes this trunk is a
single canal and again it is paired; as a rule, it is a single
vessel between two rays and paired opposite them. Hence,
from a study of the adult alone one might erroneously take

the embryonic trunk to be a single trunk that later divided
in order to encircle a ray. At frequent intervals from either
side numerous connecting branches are received from each of
the lateral veno-lymphatic sinuses. Below and immediately
behind the lateral processes of the median ventral cartilagi-
nous bar this portion of the ventral trunk received the three
terminal branches of the posterior or transverse portion of
the longitudinal hæmal lymphatic trunks (Figs. 2 and 4,
L. Hæ. T. (1)), which is a short vertical trunk formed by the
posterior union of the two longitudinal hæmal lymphatic
trunks.

From this point caudad the remaining portion of the
ventral lymphatic trunk can be designated as the posterior
portion of the ventral trunk (Fig. 4), or possibly as the
caudal trunk from its similarity to the caudal lymphatic
trunk of the Teleosts. This canal, situated immediately
below the caudal hearts, traverses the ventro-lateral border
of the median ventral bar to its distal end, where it receives
a large anastomosing branch from each lateral sinus. Like
the anterior portion, it is a single trunk between two rays and
paired in the region of the rays. It also receives many com-
municating branches from the lateral sinuses. In the region
of the anterior portion of the caudal hearts one or two
branches (Figs. 1 and 4) are given off dorsad to communicate
with each caudal heart, and each orifice is guarded by two
valves opening into the heart (Fig. 1, Val. (1).) In the case
of a heart receiving but one communication from the posterior
portion of the ventral trunk, there was always noted in all
specimens examined a ridge in the floor of the heart where
one would expect the posterior communication with the
ventral trunk to occur; possibly this may represent fused
valve-folds that had formally guarded an orifice of an
embryonic ventral trunk communication.

Longitudinal Hæmal Lymphatic Trunks (Figs. 2, 3,
4, and 4A, L. Hæ. T. or R. and L. Hæ. T.).—Within the hæmal
canal there is a pair of large lymphatic canals situated on
either side of the caudal artery and between the caudal artery

and the caudal vein. Concerning their size, each exceeded
the calibre of the caudal artery. In the region of the lateral
process of the median ventral cartilaginous bar the paired
trunks unite in a common stem (Figs. 2, 4, and 4 A, *L. Hæ.
T.* (₁), which soon bends ventrad between the two forks of
the caudal vein, and when immediately above the narrow re-
stricted portion of the ventral bar that is directly behind the
lateral processes it trifurcates. Two lateral branches (Fig. 4 A.
L. L. Hæ. T. ₁) continue ventrad across the lateral
surface of the narrow restricted portion of the median
ventral bar immediately behind the lateral processes ;
while an anterior branch (Fig. 4 A. *A. L. Hæ. T.* ₁ passes
at first cephalad and then ventrad to curve around in
front and between the lateral processes of the median ventral
bar, where it receives the ventral or inferior longitudinal
hæmal lymphatic trunk (Fig. 4 A. *V. L. Hæ. T.*), and at
this point of union there is a reservoir of considerable size,
which to a considerable extent in the 10 cm. series (but not
at all in the 85 mm. series) overlaps the sides of the lateral
processes. Immediately below the lateral processes and the
restricted portion of the median ventral bar these three
branches reunite in a common stem, which terminates in the
ventral veno-lymphatic trunk directly in front of the caudal
heart. A transverse section (as Fig. 3) sometimes shows one
of the longitudinal hæmal lymphatic trunks to be paired,
while another will show it single.

Throughout its course the longitudinal hæmal lymphatic
trunks receive at regular intervals the intersegmental
hæmal lymphatic trunks (Figs. 3 and 4 A. *Hæ. T.*) coming
up from between each set of myotomes. They always accom-
pany a corresponding artery or vein, sometimes lying in front
of it and again behind. As a rule between one pair of
myotomes there would be an artery and a lymphatic vessel,
and between the next pair a vein and a lymphatic vessel ;
there being, then, double the number of lymphatics as arteries
or veins, or the same number as arteries and veins
taken together. In case of some of the posterior hæmal

lymphatic vessels (see Fig. 4 A) these vessels were found to terminate ventrad in the great lateral sinus, and on the level with the top of the mucous sacs they sent inward a branch to communicate with the ventral or inferior longitudinal hæmal lymphatic trunk.

Ventral or Inferior Longitudinal Hæmal Lymphatic Trunk (Fig. 4 A, *V. L. Hæ. T.*).—Is a rather conspicuous vessel, though considerably smaller than the superior longitudinal hæmal lymphatic trunk. It is situated midway between the caudal vein and a longitudinal row of mucous sacs. I have not studied this trunk anteriorly, but posteriorly it terminated in the anterior fork of the common longitudinal hæmal lymphatic trunk directly in front of the lateral processes of the median ventral cartilaginous bar. The short distance in which I have observed this trunk shows it to be much larger posteriorly than it is anteriorly; hence the flow of lymph is probably caudad. Small communicating vessels have already been described coming from the intersegmental hæmal vessels. The main branches received, however, are segmental vessels that follow the surface of the anal or ventral fin-rays between every two mucous sacs and communicate ventrad with the ventral veno-lymphatic trunk. These vessels are not lettered, but are clearly shown in Fig. 4A, and may be simply dorsal continuations of the ventral or anal fin-ray vessels. Posteriorly in the 20 cm. series a direct connection with the superior longitudinal hæmal trunk was noted together with several communications from a rather large sinus situated on the lateral surface of the last two mucous sacs.

This reservoir (Fig. 4 A, *d.*) is quite conspicuous in the 10 cm. series, for it covers a large part of the lateral surface of the last two mucous sacs. Dorsad it has at least two connections with the inferior longitudinal hæmal lymphatic trunk, cephalad it ends in rather loose lymphoid tissue, ventrad it has several communications with the great lateral sinus, and caudad it tapers down into a rather long stem that terminates in the ventral veno-lymphatic trunk at the junction of one of its connections with the lateral sinus. No similar

sinus was observed in the 85 mm. series, and how many of them occur in the 10 cm. series I am unable to state, since I have not sectioned an adult anterior of the last two mucous sacs.

Dorsal Veno-lymphatic Trunk (Figs. 1 and 4, *D. V.*).— Has much in common with the ventral veno-lymphatic trunk already described. In a transverse section (Fig. 1) it will be seen in a median line, between and a little above the level of the dorsal end of the myotomes. In the region of the caudal hearts it is situated directly above the median dorsal cartilaginous bar; immediately in front of the caudal hearts its position becomes more dorsad of this bar, and anterior of the dorsal fin it travels as a sinus in the centre of a connective tissue septum that joins the two great lateral muscles. Its cephalic termination I have not determined, but posteriorly it terminates, like the ventral trunk, at the distal end of the caudal cartilage by sending off a large anastomosing branch to each lateral sinus. Also, like the ventral trunk, it is paired in the region of a cartilaginous ray and a single trunk between two rays, and throughout it sends off many connections to both lateral sinuses. The dorsal veno-lymphatic trunk is undoubtedly homologous to a similar trunk found in Scorpaenicthys and Lepisosteus (Lepidosteus), more closely allied to the latter, but less significant, in that it does not receive branches from the dorsal fin; these in Polistotrema terminate in the lateral sinuses.

Lateral Veno-lymphatic Trunks or Sinuses (Figs. 1 and 2, *R.* and *L. L. S.*).—The enormous sinus-like condition of these trunks in Polistotrema is very different from anything found in the Selachians, Ganoids, or Teleosts, but is suggestive of the lymphatic sacs of the Amphibia. If the entire region drained by the lateral trunks and their inter-segmental branches in fishes was united in a common sinus covering the entire outer surface of the myotomes, and collecting the branches from the median fins, we would have a condition similar to that found in Polistotrema.

The limits of this sinus may be described as occupying

most of the space between the lateral body muscles and the
skin, and extending from one end of the body to the other.
Cephalad its ending has not been determined, but in the tail
it terminates blindly, immediately behind the caudal plate.
Dorsad and ventrad both sinuses have many communications
with the dorsal and the ventral veno-lymphatic trunks, and
through the latter its contents reaches the caudal hearts and
the caudal vein. Ordinarily, in section (Fig. 1), one of the
lateral sinuses is much wider than the other, which is likely
due to the fact that it contains more blood or lymph.

Relatively there are not nearly as many corpuscles in the
lateral sinuses of the 20 cm. series as in the 85 mm. and still
younger embryos. In the 85 mm. series one finds the con-
nective tissue in places outside the lateral sinuses completely
filled with red corpuscles, having the appearance of germina-
tive areas.

From all the median fins numerous sinus-like branches are
received by both lateral sinuses. The so-called dorsal fin-
ray canals (Fig. 1, *D. S.*) come from the dorsal fins, the
ventral fin-ray canals (Fig. 1, *V. S.*) from the ventral or
anal fins, and the caudal fin-ray canals from the caudal
fins.   All of these vessels very closely resemble one another in
that they more or less envelop a fin-ray, traverse it from its
distal to its proximal end, and terminate in either the right or
the left lateral sinus.   Each gives off many branches to
anastomose with the adjacent fin-ray canals (Fig. 1), thus
forming a very coarse network of vessels in the fin, which, so
far as could be determined, had no connection with the arteries.

So far as was observed nearly all of the corpuscles found in
the veno-lymphatics and in the connective tissues outside were
red corpuscles, it being necessary to look some time to find a
leucocyte. In fishes, where death usually occurs after a violent
struggle, I have attributed the finding of red corpuscles in
the lymphatics to the rupturing of the delicate walls
separating them from the blood-vessels.   Prof. Carlson
informs me that an examination of the lymph of mammals
that have died from violent deaths also resulted in the finding

of blood in the lymphatics. But how account for the great number of red corpuscles found in embryos before any muscle-fibres were developed, and for older embryos and adults where a great proportion of the corpuscles were red ? It has already been stated that no direct corrections have been found between the arteries and the veno-lymphatics ; that the veno-lymphatics of the adult, especially the lateral sinuses, contain very few corpuscles compared to the late embryos, although the red greatly predominate ; that there are a multitude of red corpuscles apparently being formed in the connective tissue surrounding the lateral sinuses of the late embryos. Is it not, therefore, more than likely that in embryos the veno-lymphatics, especially the lateral sinuses, function as reservoirs or receptacles to hold the blood-corpuscles germinated in the nearby connective-tissue mesenchyme, and that the corpuscles migrate through the loose endothelial cells of the lateral sinuses, where many of them are seen undergoing cell division ?

There can be no question but that the dorsal, lateral and ventral veno-lymphatics and the caudal hearts of Polistotrema are the homologue of similar structures found in Teleosts, Ganoids and Selachians.

III. Late Embryonic Condition of the Blood-Vessels.

In all of the good series, which began with two 20 mm. embryos, the caudal artery and vein were well formed, hence only a guess can be made at the manner of their formation. In the 27 mm. series, which was carried further cephalad than the others, I found in a section taken through the hind gut that the caudal vein was in direct connection with the splanchnic blood-vessels of the yolk-sac. This may indicate a similar origin of the two, and from my point of view would expect the splanchnic vessels of the yolk-sac to arise as they do in the chick blastoderm by the union of certain blood islands or mesenchymal spaces, and the transformation of the border mesenchyme into endothelium and the enclosed cells into red corpuscles.

In a very much decomposed embryo of 15 mm. the caudal artery and vein were present, but in a similar series of 10 mm. there was no trace of them.

As in the adult the caudal artery and vein in the 20 mm. embryo separated into right and left branches above a thickening of the mesenchyme that is forming the lateral processes of the median ventral cartilaginous bar. Each fork of the caudal artery (Figs. 5, 6, and 7, *R.* and *L. Cau. A.*) passes caudad immediately under and to the side of the notochord, at first along the side and further caudad above the level of the embryonic median ventral cartilaginous bar. The two caudal veins (Figs. 5, 6, and 7, *R.* and *L. Cau. V.*) follow at first obliquely ventrad across and then caudad along the ventro-lateral surface of the embryonic median ventral bar to a point in the 20 mm. series B (Fig. 6), 150 microns from the tip of the spinal cord or 290 microns from the tip of the tail. Here on the left side, and about the same place on the right side, the caudal vein and artery bend toward each other and unite, forming one continuous cross vessel on either side near the tip of the tail. Fig. 6 shows that there is in the anastomosing portion of the caudal artery and vein several large masses of mesenchyme (*P.*). These partitions were also found throughout the entire posterior portion of the caudal vein, including the region of the future caudal heart, of not only this 20 mm. series, but in another, series A, and in the 25 mm. series (Figs. 7 and 9, *P.*). This means, then, that this portion of the vein contains the remains of large mesenchymal cavity partitions that have not disintegrated at this stage. In the 25 and 27 mm. embryos the caudal artery and vein, on either side of the tail, were likewise found to be in direct connection a little cephalad of the ending of the notochord, but in all of the later stages this early connection is lost, and the caudal artery and vein extend further caudad and break up or receive small branches coming from, or going to, the caudal fin.

Laterad and ventrad of the points of union of the caudal artery and vein in the 20 mm. series there is nothing but

undifferentiated mesenchyme. No trace of the embryonic myotomes or lateral sinus mesenchymal spaces occurs until a section 210 microns cephalad of the tip of the spinal cord is reached.

Intersegmental Blood-vessels.—As previously stated in the adult under the head of the intersegmental or hæmal lymphatics there are twice as many intersegmental lymphatics as there are arteries or veins—that is, the arteries alternate with the veins in traversing the septa between the myotomes; while each septum would have a lymphatic vessel, it would have either an artery or vein, not both. A typical interseg- mental artery upon leaving the lateral side of the caudal artery almost immediately divides into a dorsal and ventral branch. In the course of the dorsal branch, it gives off lateral branches to the myotomes and a median branch to the neural canal; while the ventral branch passes ventrad along the inner surface of the septum between two myotomes, supplying each. The course of the intersegmental veins is practically the same as the arteries, except that the dorsal and ventral veins terminate separately at different levels on the lateral surface of the caudal vein instead of first uniting in a common stem. The veins, then, are apparently in a more primitive condition than the arteries.

It is not my intention to take up the development of these vessels only in so far as they are concerned in the formation of the caudal hearts, but in order to get a true conception or typical state of these vessels in the embryo it is necessary to examine them in front of the point of bifurcation of the caudal vessels.

In the 20 mm. series B, it will be seen at a glance that the intersegmental arteries are much further advanced than the veins, which have barely started. It will also be noted that the caudal artery and vein have assumed different shapes; the artery is very much depressed, having a very great lateral diameter and almost no dorso-ventral diameter, while the caudal vein is almost round. Throughout the whole length of the caudal vein there are on either side one or two.

mesenchymal cavities, which for the most part have gained connection with the vein and with each other. They are lined with an undifferentiated mesenchymal endothelium, which cells in many cases have lateral processes that cross these cavities as well as line them. In a later stage, 27 mm., the mesenchymal walls between these cavities and the caudal vein have broken down, thereby greatly increasing the lateral diameter of the vein. From these cavities both in the 20 and the 27 mm. series short dosal and ventral intersegmental branches were given off; these for the most part ended peripherally in more or less of a cord of cells, indicating that the origin of the intersegmental veins is from sprouts off from the caudal vein. Even in these small embryos the intersegmental arteries have attained the course already described for the adult. In the 27 mm. embryo the median branch to the spinal cord is more clearly defined than in the adult, and in both series the intersegmental arteries travelled much further laterad before dividing than they did in the adult.

In the region of the caudal heart and behind the caudal heart in the 20 mm. series A, which is not as far advanced as series B, all of the intersegmental vessels are in an embryonic condition. The caudal intersegmental vessels are more numerous than intersegmental vessels should be; those in the caudal heart region appear to be about twice the normal number and to increase as you go caudad. Nowhere in this region do you find a typical intersegmental artery arising from the lateral surface of the caudal artery and dividing into dorsal and ventral branches, but rather each branch takes its origin from dorsal or ventral surface of the right or left caudal artery (Fig. 7, *Ints. A.*) and then passes latero-dorsad or latero-ventrad. In the region of the caudal heart there are at least three latero-dorsal outpocketings, which have every evidence of being embryonic intersegmental veins (Fig. 7, *Ints. V.*). They at first passed laterad, then dorsad, and upon reaching a height about on a level with the left caudal artery, but considerably laterad of it, they end blindly. The second of these embryonic veins in the heart region (Figs. 7 and 14, *Ints. V.*)

anastomosed with a corresponding ventral intersegmental artery, and several such communications were observed further caudad. No embryonic ventral intersegmental veins were found in the caudal region. From the lateral reconstruction (Fig. 7) several of the larger mesenchymal spaces (C.) might readily be taken for embryonic dorsal intersegmental veins, but transverse sections demonstrate their position and course to be too far mesad to be considered as such.

In the 20 mm. series B, 25 mm., 27 mm., and 60 mm. series there are no embryonic intersegmental veins in the region of the caudal heart, nor is there any evidence in these series of a degeneration of the embryonic veins shown in the 20 mm. series A. Also there is absolutely no reason for supposing that the degenerating endothelium of these intersegmental veins formed the cavities (C. and c.), in the reconstructions 6 to 10, for not only is their position and irregular arrangement against it, being located too far laterad, but instead of these spaces in the mesenchyme being lined with flattened endothelium they are bordered by undifferentiated mesenchyme. In the 20 mm. series B, 25 mm. and 27 mm. series these intersegmental veins have survived behind the caudal heart and are numerous. Posterior of the caudal heart in the 20 mm. series B, the embryonic intersegmental veins resemble the embryonic intersegmental veins of the heart region in the more embryonic 20 mm. series A, and in several places the veins were seen to anastomose with the arteries. Nowhere in the caudal region of the 20 mm. series B were any embryonic intersegmental veins given off ventrad; though in the 27 mm. series they were in the process of formation, apparently as sprouts from the caudal vein. In all of the embryos these embryonic intersegmental veins had at first a lateral course, and then bent dorsad or ventrad, being situated some little distance from the notochord, for the most part too laterad, to take any part in the formation of the so-called caudal heart mesenchymal spaces (C. and c.). No trace of well-formed intersegmental veins emptying directly into the heart, as have already been described for the adult, were seen until the

85 mm. series was reached. Here in the extreme posterior ventral corner, a ventral intersegmental vein pierced the m. cordis caudalis and terminated in the left caudal heart through a very small orifice. This vein I took to be the homologue of a more anterior vein in the 20 cm. series, which emptied into the anterior end of the heart at about its median line. This would indicate, then, that the permanent intersegmental veins emptying into the caudal hearts are of late formation, beginning somewhere in embryos of between 60 and 85 mm. in length.

As regards the intersegmental arteries in the caudal heart region, many of them in the 20 mm. series B, 25 mm., 27 mm., and 60 mm. series have retained their primitive condition, as in 20 mm. series A, of having dorsal and ventral intersegmental arteries, arising separately from the caudal arteries; while in some instances, as in the 27 mm. embryo, the basal parts of these arteries have united in a common stem (Fig. 10, *Ints. A.*). This part of the process of the development of the intersegmental arteries of Polistotrema may be, then, somewhat analogous to the spinal ganglion cells in the mammalian embryo changing from bipolar to unipolar as development advances.

IV. DEVELOPMENT OF THE CAUDAL HEARTS IN POLISTOTREMA.

In considering the mode of formation of the caudal hearts in Polistotrema the condition of the caudal hearts in each of the various stages will be discussed, beginning with the youngest. Of these there are two well-preserved embryos of 20 mm. in length, which have been designated as series A and B; although of equal length, practically all of the structures are considerably more advanced in series B.

20 MM. POLISTOTREMA SERIES.

In both series A and B, the caudal hearts are shown by lateral reconstructions of the left embryonic caudal heart (Figs. 6 and 7) and by several transverse sections (Figs. 11 to

14) to be in very early stages of formation. In fact, the caudal vein itself may not have received its maximum size in this region any more than it has further cephalad or caudad, but since in the adult the caudal hearts are nothing more than large expansions of the anterior portion of the right and left branches of the caudal vein, it is impossible in the embryo to discriminate or define any boundaries between the enlargement of the caudal vein such as has been described both anterior and posterior of the caudal hearts from the normal development of the caudal hearts, for both take place in exactly the same manner.

A glance at the embryonic caudal heart in the reconstructions 6 and 7 demonstrates that the original left caudal vein (*L. Cau. V.*) is bounded, more or less on all sides, by certain mesenchymal spaces, from the region of the embryonic lateral processes of the median ventral cartilaginous bar to a point designated by *Z.*, denoting the posterior extremity of the caudal heart as determined by its relation to the spinal nerves. These spaces can readily be separated into two distinct types: first, mesenchymal spaces (*C.*) that have gained connection with the caudal vein, and second, mesenchymal spaces (*c.*) that have not at this stage obtained connection with the caudal vein.

For the sake of maintaining a logical order the small isolated mesenchymal cavities (*c.*), that have not at this stage gained connection with the caudal vein, will be considered first. As will be shown later they are undoubtedly the first cavities to appear. In the two reconstructions they are found everywhere where the heart is in the process of formation, and in the extreme posterior end of the embryonic caudal heart in reconstruction 6, which is the region of the heart last to form, they were the only cavities observed. One of these cavities is shown in sections 11 and 13; the former is taken through an isolated cavity in the posterior end of the embryonic caudal heart and the latter is from the anterior end. In both cases these cavities are small, but well-defined, not artifacts, and are bounded simply by the adjacent

mesenchyme, not at this stage differentiated into flattened endothelium.

In certain areas, as may be designated by the red corpuscle (*R. C.*) in Figs. 12 and 13, there are places which appear to be the very beginnings of the vacuolation of the mesenchymal spaces just described. This process as shown from the examination of a number of isolated mesenchymal spaces is apparently nothing but a degeneration or atrophy of certain of the mesenchymal cell processes and the thickening of others to form the boundaries of the cavity; while some of the enclosed cells may enlarge, become round and develop into red corpuscles before the mesenchymal spaces become connected with the caudal vein.

Concerning the mesenchymal spaces (*C.*) which have already gained connection with the caudal vein, there are several distinct stages illustrating different periods of growth of the larger cavities that take such a conspicuous part in the excavating of the caudal hearts out of the mesenchyme. Of these an early, middle, and a late stage will be described with considerable detail. All of these stages are shown in outline in the reconstructions 6 and 7, while some of them may be seen to advantage in transverse sections.

The earliest stage of one of these connected mesenchymal spaces (*C.*) is well illustrated by the dorsal cavity to the right in Fig. 12. When compared to the isolated mesenchymal space (*c.*) in Fig. 11 it will be found to be identical, except that the mesenchymal lining or undifferentiated endothelium of the vein has broken down, joining the cavity with the vein. Observe in Fig. 12 some of the remains of the original partition at the orifice of the mesenchymal space. The character of this orifice and all similar mesenchymal space orifices opening into the caudal vein is strongly against the hypotheses that these spaces are out-pocketings or sprouts from the caudal vein, as is also the character of their walls. Immediately to the left of the above described mesenchymal space in Fig. 12 there is a slightly larger but identical connected mesenchymal space shown, only in this section its

extreme distal end is shown, which is the region of future growth, and the manner of growth by the breaking down of the mesenchymal cell processes is evident from this section. Opposite line 12 in the reconstruction 6; the above cavity (*C.*) is shown to have attained anterior connection with the left caudal vein.

Two dorsal connecting mesenchymal cavities (*C*) in the reconstruction 7 are typical examples illustrating a median stage of the development of a larger mesenchymal cavity that later becomes a portion of a caudal heart. These cavities simply represent one of the earlier connected mesenchymal spaces described in the previous paragraph as having united with one or more distal isolated mesenchymal spaces, thereby considerably increasing the size of the former. It might be surmised from an examination of the lateral reconstruction 7 that these spaces are developing intersegmental veins or capillaries, but their position in transverse section is shown to be too far median for them. In fact similar cavities are found median of the caudal vein—a region never penetrated by capillaries or intersegmental veins.

The third stage of development of the large connected mesenchymal cavities that contribute to the formation of the caudal hearts is simply a more advanced state of the middle stage previously described. It might be said to consist of several isolated mesenchymal spaces that have united with a larger connected mesenchymal space at either or both ends, and which brings these cavities in direct communication with the right or left caudal vein. Such a stage as this, or even a more advanced one, where all of the mesenchymal spaces have coalesced in one large cavity which is in direct connection at both ends with the caudal vein, are shown in the reconstructions 6 and 7. In Fig. 13 one of these cavities (*C.*) is shown in section opening into the left caudal vein, and in Fig. 12 two of these cavities (*C.*) appear ventrad of the right and left caudal vein. Since they are cut through different planes they will illustrate very well two different areas of one of the large cavities in the third stage of development. In

the floor of the left caudal vein will be seen (Fig. 12, *C.*) one of
these cavities opening into the vein ; observe that the orifice
bears evidence of the breaking down of the partition
separating the cavity from the vein.   In tracing this cavity
cephalad it was found to open into three smaller mesenchymal
spaces almost identical to the three mesenchymal spaces (*C.*)
of another cavity of the third stage, shown below the right
caudal vein in the same figure, and when followed further
cephalad the mesesenchymal spaces terminated in a large
cavity that emptied into the left caudal vein from below.   A
little anterior of the cavity described above there is another
large connected cavity of the third stage, not shown in any
of the transverse section figures, but almost identical to the
cavity previously described.   It has, however, one striking
difference, namely, the process of the union of its mesen-
chymal spaces has progressed further ; while the mesenchymal
walls separating these mesenchymal spaces in the first cavity
were quite thick, resembling the condition found in the
ventral cavity below the right caudal vein in Fig. 12 ; in this
more advanced cavity they are reduced to two parallel
mesenchymal cell processes, separating three median-lateral
mesenchymal spaces from each other.   These two remaining
processes consist of the union of two ventral processes from
two mesenchymal cells, now situated as border-cells on the
dorsal ˏside of the future mesenchymal cavity with two
similar dorsal processes from two mesenchymal cells located
on the ventral border of this future mesenchymal cavity ;
while from all of these mesenchymal cells there are median-
lateral processes, which will help constitute a sort of un-
differentiated endothelial lining for this cavity.   It would
require but the atrophy of these delicate protoplasmic pro-
cesses to convert the three mesenchymal spaces into one large
mesenchymal cavity that would be connected at both ends
with the left caudal vein, and in like manner the disintegra-
tion of the partition separating this cavity from the vein
would result in the excavation of a considerable portion of
the caudal heart in the ventro-cephalic region.

Now the question might be asked, What becomes of the mesenchymal cells in the walls of the mesenchyme separating one space from another, as, for example, in Fig. 12, the ventral mesenchymal spaces (*C.*), when they coalesce to form one large cavity ?   It is quite certain that some of these cells are becoming round and increasing in size preparatory to becoming red corpuscles, while the border cells are apparently flattening to a slight extent in order to become endothelium for the caudal heart.   It is doubtful, however, if all the cells take one or the other of these two courses.   There remains, then, the processes of disintegration and migration as possibilities.

In connection with the process described above of an isolated mesenchymal space becoming in a later stage connected with the caudal vein or becoming a connected mesenchymal space as it was styled, the question might be asked, How do you know that this change actually occurs without being able to demonstrate it experimentally ?   In reply it might be said that it would be absurd to consider these well-defined mesenchymal spaces as artifacts, for if artifacts, they would occur in the mesenchyme outside the limits of the developing caudal hearts, blood-vessels, and lymphatics.   In the immediate region of the caudal heart they are found only in the region of the small connected mesenchymal spaces and the larger mesenchymal cavities that unite with each other and the caudal vein to form the caudal heart.   At the orifices of a great many of the small connected mesenchymal spaces and the larger mesenchymal cavities there are left the remains of the partitions that once separated these spaces or cavities from the vein, which is evidence against the hypothesis that they arose as sprouts or out-pocketings from the vein, as is also the fact that these cavities are lined with mesenchyme and not differentiated endothelium.   Not many of these cavities have sufficient regularity to be the remains of intersegmental veins.   In fact, they occur mesad of the caudal vein, a region never traversed by intersegmental veins or capillaries.   The isolated mesenchymal spaces and the more

distal or growing areas of the larger mesenchymal cavities apparently furnishes us with the solution of the origin and manner of growth of these mesenchymal spaces and cavities. Here in section may be seen the remains of what has transpired. The protoplasmic processes of the mesenchymal cells in the centre were found to be disintegrating and increasing in diameter on the border of the cavity, while some of the enclosed mesenchymal cells had increased in size, and were becoming spherical to form the red corpuscles, and others in the walls of the larger cavities had flattened in places to form a part of the lining for the future caudal heart.

Another possible source of a very small portion of the caudal hearts in Polistotrema is shown in the 20 mm. series A to be from the proximal ends of three embryonic intersegmental veins (Fig. 7, *Ints. V.*). These embryonic vessels have already been described under the head of intersegmental veins, and no trace of these vessels was found in the 20 mm. series B or any of the later series until the 85 mm. series was reached, indicating that the persisting intersegmental veins of the adult did not arise in the caudal heart region until a stage midway between 60 and 85 mm. The anterior and posterior of these so-called embryonic intersegmental veins in the 20 mm. series A (Fig. 7, *Ints. V.*) are in every way comparable to the intersegmental veins that are forming anterior and posterior of the caudal heart region. Tracing them from the dorso-lateral surface of the left caudal vein they pass laterad for some little distance, and then bend dorsad to end blindly, about on the level with the left caudal artery. If, then, these are true embryonic intersegmental veins, arising as sprouts from the left caudal vein, it would be possible for their proximal portions to contribute slightly to the formation of the left caudal heart. Concerning the middle vessel, which has been described as an embryonic intersegmental vein anastomosing with a corresponding intersegmental artery, is shown in transverse section (Fig. 14, *Ints. V.*) to bend dorsad much sooner than the other embryonic intersegmental vein did. It is possible, then, that this vessel is an embryonic

intersegmental vein that had been intercepted in its growth by the crossing of a ventral intersegmental artery, or it may have been only a capillary, or possibly a caudal heart mesenchymal cavity that had gained connection both with the caudal vein and an intersegmental artery. At any rate, granting it to be an embryonic intersegmental vein, it could have but little to do, relatively, with the formation of the left caudal heart. The mesenchymal space (Fig. 14, C.) opening into the so-called embryonic intersegmental vein has every indication of being an isolated caudal heart mesenchymal space that has acquired connection with the so-called embryonic intersegmental vein, rather than being a sprout from the vein. It will be seen, then, that the intersegmental veins or capillaries, which, according to Lewis, Baetjer, Huntington, and Miller, take such an important part in the formation of the lymphatic hearts in mammals, birds, and reptiles, takes but a very insignificant, if any, part in the formation of the caudal hearts in Polistotrema.

Both of the reconstructions 6 and 7 demonstrate that the left embryonic caudal heart has at this stage received no communication from the ventral veno-lymphatic trunk. In series B, reconstruction 6, the ventral veno-lymphatic trunk is represented by at least one large mesenchymal cavity ($l.\ v.\ v._{(1)}$), which is about to gain connection with the embryonic caudal heart through the breaking down of the thin wall separating them. Also but little has been accomplished toward the differentiation of an endothelial lining, other than the thickening of the cell-processes lining the cavity and the slight elongation of some of the cells; it would be extremely difficult, if not impossible, to identify one of the typical dorsal or ventral border cells of the heart cavity from a typical mesenchymal cell. There is a marked massing of the mesenchyme to form the connective-tissue layer of the heart, but no differentiation of mesenchyme into connective tissue.

25 AND 27 MM. POLISTOTREMA SERIES.

A glance at fig. 9 will demonstrate that the left embryonic caudal heart (*l. cau. h.*) of the 25 mm. embryo has made considerable progress over both of the 20 mm. embryos. The anterior ventral region has been completely excavated, as has also the anterior dorsal region, except in the latter there is left traces of the mesenchymal partition that formally separated two of the larger cavities from each other or a cavity from the vein. The middle and posterior portions of the heart have not received their maximum dorsal growth at present, and the mesenchyme above and to either side of the original left caudal vein is invested by a number of large connected mesenchymal cavities and several isolated cavities, while the extreme posterior end of the caudal heart, namely, that portion located in front of the line *Z.*, is made up solely of the original caudal vein surrounded on all sides by isolated mesenchymal spaces (*c.*).

Anteriorly in the dorsal two thirds of the 25 mm. embryonic caudal heart the endothelium is much further differentiated than in the previous series, which means that its cells are somewhat more flattened, and their distal processes that formally extended out into the mesenchyme are lost, but ventrad, throughout the entire heart the border-cells are nearly round, very abundant, with almost no processes, and in a state where they could be easily moved. It would be impossible to discriminate between the ventral border-cells of the heart and the undifferentiated mesenchyme more distad.

There are no intersegmental veins emptying into the 25 mm. caudal heart unless the extreme proximal or caudal vein portion is considered as part of the heart; if so, the caudal heart would receive a dorsal and a ventral intersegmental vein (Fig. 9, *Ints. V.*). It will be seen that the first or anterior mesenchymal cavity of the left embryonic ventral veno-lymphatic trunk (Fig. 9, *l. v. v.* $_{(1)}$) has gained connection with the anterior ventral corner of the left caudal heart, but no valves have appeared at the orifice (*O.*). Three other mesenchymal

cavities belonging to the left ventral trunk have also appeared in a row behind the first (Fig. 9, *l. v. v.* $_{(2)-(4)}$), and above the third and fourth, which have coalesced, the mesenchyme is vacuolating, indicating the place ($O._2$) where the second or posterior communication with the heart is likely to occur.

Reconstruction 10, which is from a 27 mm. embryo, shows a marked advance in the development of the caudal heart over the previous 25 mm. embryo as seen in reconstruction 9. Anteriorly the caudal heart had reached practically adult conditions so far as relative size was concerned, but from its centre caudad it consisted of a number of very large connected mesenchymal cavities (*c.*), which have occurred and assumed lines of growth in two different directions. The most anterior path or chain of these mesenchymal cavities had assumed a general caudal direction from the dorsal central part of the heart; while the posterior chain of the mesenchymal cavities had taken a dorsal course, considerably posterior, from the left caudal vein. A glance at Fig. 10 will show that the distal ends of these two different lines of growth of mesenchymal spaces, for the most part connected, were about to anastomose, and when this is accomplished the general outline of the heart will be completed, excepting that its dorsal boundaries will be expanded somewhat, and it will be added to posteriorly by the formation and union of a few additional mesenchymal spaces. These posterior isolated cavities were not present in this series, but were found in all of the later stages. As a result of the meeting and union of these two chains of mesenchymal cavities decribed above, there would be left in the centre an island of mesenchyme, identical to an anterior island (Fig. 10, *P.*) already formed, which in due time would be eliminated from the heart. From the lateral reconstruction 10, the posterior chain of mesenchymal cavities has the appearance of a developing intersegmental vein, but transverse sections reveal its position to be too far median— in fact it arises from the dorsal surface of the vein, and its course is dorsad close to the embryonic median ventral cartilaginous bar. Had not earlier stages of the caudal heart

region been examined, these two chains of more or less con-
nected mesenchymal cavities might readily have been taken
for out-buddings from the vein and the caudal heart, and the
caudal heart been described as being formed from sprouts
from the caudal vein.

The endothelium and connective-tissue layers were found
to be in about the same state as in the previous 25 mm.
embryo and no intersegmental veins terminated in the left
caudal heart. Immediately below the anterior end of the
left caudal heart the two anterior mesenchymal cavities of the
left embryonic ventral veno-lymphatic trunk (Fig. 10, *l. v. v.*
$_{(1), (2)}$) had united, forming a large cavity, which, as in the
previous series, had gained connection with the anterior
ventral corner of the heart, and about the orifice there was
a slight massing of the mesenchyme (Fig. 15, *val.* $_{(1)}$), possibly
indicative of a very early stage in the formation of the valves
guarding this opening. Likewise the third and fourth
cavities of the embryonic ventral trunk (*l. v. v.* $_{(3), (4)}$) have
coalesced and the latter has joined the heart, thereby estab-
lishing the second or posterior communication between the
ventral veno-lymphatic trunk and the left caudal heart.

58 AND 60 MM. POLISTOTREMA SERIES.

As regards the caudal hearts in these two series, they
appear to be practically in the same stage, and since the
general preservation of the 60 mm. series is better, the des-
cription will be taken almost entirely from this series.

Except for a general dorsal expansion and a small caudal
addition, the caudal hearts of the 60 mm. series have not
increased in size relatively over the 27 mm. heart, but con-
sidering the general histogenesis of the organ, marked
progress has occurred. The posterior embryonic condition
referred to above is in the posterior dorsal end of the heart,
where both isolated and connected mesenchymal cavities
(Fig. 8, *c.* and *C.*) are present. Not only has the heart gained
anterior and posterior connections with the ventral veno-

lymphatic trunk, but conspicuous right and left valves (Fig. 16, *Val.* (1)) will be seen guarding the first or anterior orifice, and similar valves are in the process of formation at the opening of the second or posterior communication. The reason why these valves are not shown in reconstruction 8 is that the reconstruction is made through the median line of the heart, and it would therefore pass directly between the right and left valves.

Concerning the histogenesis of the layers of the heart at this stage, most any section—as, for example, Fig. 16—will demonstrate that the endothelium is well differentiated; instead of consisting of many rounded cells with rather short processes, as in the earlier series, we have here the characteristic flattened cells and long processes of the adult. Also the outer connective-tissue layer has changed from a concentrated mass of mesenchyme to a layer of fairly well differentiated fibrous connective tissue (Fig. 16, *F. C. T.*), and the m. cordis caudalis (Fig. 16, *m. c. c.*), although still in an embryonic state, is sharply marked out from the connective-tissue layer of the heart and the outer undifferentiated mesenchyme.

85 MM. POLISTOTREMA SERIES.

No figures are given to illustrate the condition of the caudal hearts in this series, which have attained an adult state, except for the extreme caudal end, which bears a striking resemblance to the 60 mm. hearts described above.

The valves and the different layers of the hearts strongly resembled the 20 cm. adult, and one intersegmental vein was found emptying into the posterior end of the left heart.

Since the rate of growth of the Polistotrema embryo is very slow, the time consumed from the beginning of the development of the caudal hearts in the 20 mm. embryo to their completion in the 85 mm. embryo, or a little later, requires, probably, several months. This, together with the primitive state of the lymphatics in the adult, should make Polistotrema a most favoured vertebrate for the onto-

genetic study of a veno-lymphatic heart. A striking diffe-
rence between the caudal heart of Polistotrema and the
embryonic sacs of the higher vertebrata is that the caudal
hearts of Polistotrema, when once formed, never lose
their primary connection with the vein, and acquire a second
communication. It was shown in the adult to be a con-
spicuous swelling of the two posterior branches of the caudal,
and to be formed after the manner outlined below.

From the previous description it will be seen that the main
process involved in construction of the caudal hearts in
Polistotrema consists, first in the formation of certain
isolated mesenchymal spaces in the region of the anterior
ends of the two branches of the caudal vein, by the breaking
down of certain mesenchymal cell processes in the centre,
and the thickening of others to form the boundaries of the
cavity. Some of the cells in the centre may become spherical,
increase in size, and eventually become transformed into red
corpuscles. The next stage results in the breaking-down of
the mesenchymal partition between this cavity and the
caudal vein. About this time other more distal isolated
mesenchymal spaces will occur. These will increase in size,
meet and unite with the above-mentioned connected mesen-
chymal cavity, and frequently the distal ends of these cavities
will coalesce in a larger cavity that will come in contact with,
and join, the caudal vein by the disintegration of the partition
separating them, thus establishing a second connection with
the vein for above-mentioned cavities. While this is being
accomplished, or shortly after, the mesenchymal walls
separating the middle spaces will become broken down,
leaving one large mesenchymal space that is connected at
either end with the caudal vein. Apparently, in places the
border mesenchymal cells of this cavity are flattening to
contribute to the endothelium of the heart, while certain of
the enclosed cells, as was noted for the isolated spaces, had,
by increasing in size and becoming spherical, differentiated
into red corpuscles. In like manner the mesenchyme on all
sides of the anterior portion of the two forks of the caudal

vein becomes honeycombed with mesenchymal spaces and cavities of the various stages described above, beginning anteriorly and gradually occurring more posteriorly. By the coalescence of the larger cavities with each other and the vein, through the disintegration of the walls separating them, the caudal hearts are excavated out of the mesenchyme, mainly from above and below the anterior portion of the two branches of the caudal vein, and the caudal hearts for the most part become lined with endothelium derived from the flattening of the boundary mesenchymal cells of the larger cavities, and not from the migration of cells originating from pre-formed endothelium.

It was shown beyond a reasonable doubt that the isolated mesenchymal spaces were not artifacts, and that they, as well as the larger connected mesenchymal cavities, frequently occur mesad of the two branches of the caudal vein— a region never penetrated by capillaries or intersegmental veins.

In the 20 mm. series A, reconstruction 7, it was demonstrated that it would be possible for the proximal ends of three so-called dorsal intersegmental veins to take a minor part in the formation of the caudal heart. It should, however, be stated that no trace or remains of these embryonic vessels were found in the 20 mm. series B or in any of the later series until the 85 mm. stage was reached. Consequently there would be just as much reason for advocating that the border cells of these embryonic vessels disintegrated or possibly reverted back to mesenchyme, from which their cells are hardly distinguishable, as to maintain that in some way they contributed to the formation of the caudal heart. Granting, then, that they did take part in the formation of the Polistotrema caudal heart, it could only be a minor factor, and not the main process as is held by Lewis, Baetjer, Huntington and Miller for the lymph-sacs of mammals, birds and reptiles.

The formation of the caudal hearts as I have interpreted them in Polistotrema is almost identical to what Stromsten

found in the turtle, and in line with the earlier work on the lymphatics, as illustrated by Budge and Sala, but at variance with a considerable recent work on the development of the lymphatic sacs in the higher Vertebrata by Miss Sabin, Huntington, McClure, Lewis, Knower, Hoyer, Baetjer, Barański, and others, who maintain that the lymph-sacs in mammals, birds, reptiles and amphibians have their origin directly from the larger veins, either as sprouts or fenestrations, or from their branches or capillaries.

V. Development of the Ventral Veno-Lymphatic Trunk.

It is my intention in this paper only to take up the manner of formation of this trunk in so far as to isolate it from the caudal hearts, with which it is very closely associated.

In a transverse section taken through the anterior end of the left embryonic caudal heart of a 20 mm. series, as, for example, Figs. 5 and 13, there will be seen some distance below the left caudal vein a conspicuous isolated cavity in the mesenchyme ($l. v. v._{(1)}$), containing several red corpuscles. If no further examination of this cavity be made, it might easily be dispensed with as an isolated ventral mesenchymal cavity that would take part in the formation of the left caudal heart. If, however, this cavity is traced further cephalad it will be found, as is shown in reconstruction ($6, l. v. v._{(1)}$), to be situated too far ventrad to take any part in the construction of the left caudal heart, and in addition it can be followed under the embryonic lateral process of the median ventral cartilaginous bar to a point within 30 microns of a vertical plane that passes through the point of bifurcation of the caudal artery, which of course is considerably cephalad of the limits obtained by the caudal heart. It will also be noted that this rather large cavity was formed by the union of two or more smaller mesenchymal spaces. This large cavity I believe to be the first, or most anterior, of a series of mesenchymal cavities that will occur later and coalesce to form the posterior portion of the ventral veno-

lymphatic trunk. At this stage it has not obtained connection with the anterior end of the embryonic caudal heart, although transverse section 13 demonstrates that the mesenchyme between the two is becoming vacuolated, indicating the place where the first or anterior communication will occur. Still further caudad in reconstruction 6 there is another isolated mesenchymal cavity ($v. v.$), which occupies a position too ventrad to take part in the formation of the left caudal heart, and more than likely represents one of the more posterior cavities of the chain of cavities that will later unite in forming the posterior portion of the ventral trunk. The 20 mm. series A portrays a still earlier stage of this most anterior cavity of the ventral trunk, which is nothing more than a small vacuole in the mesenchyme (Fig. 7, $l. v. v._{(1)}$).

The left ventral veno-lymphatic trunk in the 25 mm. series is shown in reconstruction 9 to consist of a longitudinal chain of at least four mesenchymal cavities ($l. v. v._{(1)-(4)}$). The first cavity ($l. v. v., _{(1)}$) is of considerable size, and is probably derived from the union of several smaller mesenchymal spaces. As in the 20 mm. series, it not only passed cephalad under the embryonic left lateral process of the median cartilaginous bar, but extended further cephalad to a point 30 mm. in advance of union of the two branches of the caudal vein and considerably past the point of bifurcation of the caudal artery, while posteriorly it had gained connection with the anterior ventral corner of the left caudal heart, probably through coming in contact with the heart and the breaking-down of the wall separating them. Of the three remaining cavities, the first or second in the chain ($l. v. v._{(2)}$) is still isolated, while the two last have united in a single cavity, and the mesenchyme above this cavity is more or less vacuolated, preparatory to establishing the second or posterior connection ($o._{(2)}$) with the left caudal heart.

In the 27 mm. series, reconstruction 10, the embryonic ventral veno-lymphatic trunk is represented by a longitudinal chain of at least five mesenchymal cavities. The first and second cavities have coalesced in one, which not only com-

municates above with the caudal heart, but about the orifice
there is a slight massing of the mesenchyme (Fig. 15, *val.* (1))
possibly indicative of the beginning of valves, and the
anterior ending of this cavity under the embryonic lateral
process of the median ventral bar resembles the ending of the
first cavity of the 25 mm. series. The third and fourth
cavities (*l. v. v.* (3), (4)) have also united, but the fifth
(*l. v. v.* (5)) is still isolated. While the fourth cavity has gained
connection with the left caudal heart there is at present no
trace of valves at the orifice. Both the second and the fourth
cavities have joined two isolated mesenchymal cavities
(*L. L. S. C.*) belonging to the left embryonic lateral veno-
lymphatic sinus, and the third mesenchymal cavity has
gained direct connection with the left lateral sinus, doubtless
through a union of certain of the lateral sinus mesenchymal
spaces. It might be said for both embryonic ventral veno-
lymphatic trunks of the 27 mm. series that they consist of at
least five, more or less, connected cavities situated directly
below the caudal heart ; that they have obtained connection
with both the lateral sinuses and the caudal hearts, and these
vessels have now obtained a length equal to that of the
caudal heart.

Reconstruction 8 of the 60 mm. series shows the left ventral
veno-lymphatic trunk (*L. V. V.*) to have assumed practically
adult conditions in the region of the caudal heart, with the
exception that there will be found in a few places the remains
of former mesenchymal cavity partitions that have not com-
pletely disintegrated. The orifices of the anterior and
posterior communications between the ventral trunk and the
left caudal heart are guarded by valves, well developed in the
former and in the process of formation in the latter.
Numerous connections (*L. L. S. Con.*) have been received from
the left lateral sinus, and immediately in front of the caudal
hearts the three terminal branches of the posterior portion of
the longitudinal hæmal lymphatic trunk will be found to
culminate.

VI. Formation of the Caudal Heart Valves.

As has already been pointed out in the 27 mm. series there is a slight massing of the mesenchyme (*val.* $_{(1)}$) around the orifice of the first or anterior communication between the left ventral veno-lymphatic trunk and the left caudal heart, which may be the forerunner of two valves that will occur later. It is not, however, until the 58 and 60 mm. series are reached that any real clue is obtained as to the manner of the formation of these valves.

In the 60 mm. series the right and left valves (Fig. 16, *Val.* $_{(1)}$) guarding the orifice of the first or anterior communication between the left ventral veno-lymphatic trunk and the left caudal heart are conspicuous and fairly well formed. They are composed of masses of mesenchyme or of but little differentiated connective tissue, which has migrated dorsad and inward into the heart from the right and left walls of the ventral trunk's connecting vessel. A close inspection of their structure will disclose that, notwithstanding that they belong to the same outer connective-tissue layer of the heart and ventral connecting vessel, yet their differentiation into connective tissue has not advanced nearly as far. The valves guarding the second or posterior communication of the ventral trunk with the left heart are in a much earlier stage of formation, in fact, only the very beginnings of embryonic connective-tissue valve-folds are visible on either side of the orifice. What appears to be an intermediate stage is shown at the orifice of the first or anterior communication of the ventral trunk with the right caudal heart. Here the right and left valve-folds of but little differentiated connective tissue from the side walls of the communicating vessel have grown inward and dorsad, and have fused irregularly, so that in places there would be a solid wall of tissue, and in other places temporary openings. Fig. 17 shows a region of these valve-folds where there is an opening on the left side, while in the next section cephalad (Fig. 18) there has been a complete fusion of the valve-folds, so that there is no orifice, and

in the next section cephalad a temporary opening is present, but on the right or opposite side to Fig. 17. Just how the permanent valves are constructed out of this rather coarse sieve-like valve-fold, or why these valve-folds should fuse at all, my material does not demonstrate, but would expect the permanent opening in the centre to occur by the gradual disintegration of the tissue in that region. Transverse sections apparently show that this valve-fold extends dorsad into the heart, cephalad and caudad of the immediate regions of the anterior and posterior ventral orifices.

A still later stage, as shown in the 85 mm. series, demonstrates considerable change in the valves guarding the ventral orifices of the heart both as regards form and general structure. Instead of appearing short and broad in section, as in Fig. 16, they have become long and slender bands of well-differentiated connective tissue, lined with endothelium. As was observed in the 60 mm. series the second or posterior valves are considerably less advanced than the first or anterior pair. In the 85 mm. series the posterior pair are in about the same stage as the anterior pair in the 60 mm. series.

VII. HISTOGENESIS OF THE MUSCULI CORDIS CAUDALIS.

In discussing the origin of this muscle it will be necessary to begin with the myotomes, and throughout a comparison of the two is of interest on account of their close relationship both as regards position and innervation.

If a comparison be made of the dorso-ventral extent of the myotomes in the 20 mm. series (Fig. 5, *myo.*) and an 85 mm. stage (Fig. 1, *Myo.*) in which adult conditions are practically reached, it will be seen that the embryonic myotomes in the 20 mm. series must grow considerably, both dorsad and ventrad, before their relative adult dimensions are reached. A higher magnification reveals the central part of the myotome in the 20 mm. series to be much further advanced than is either the dorsal or the ventral end. Here the myoblasts (Fig. 21, *Myo. Myob.*) show the beginnings of

fibrillæ, and the mesenchyme is migrating inward in places to form the internal perimysium (*i. per.*), while both the dorsal and the ventral ends consist only of a mass of undifferentiated myoblasts and mesenchyme, which are continuous with dorso-median and ventro-median masses of greatly thickened mesenchyme. In our study the ventro-median mass of concentrated mesenchyme (Figs. 5 and 19) are of especial interest. It can be traced inward and ventrad to the lateral wall of the caudal heart, and below the heart to be continuous with another conspicuous concentration of mesenchyme that is situated between the two caudal hearts, and is destined to form the membranous stage of the median ventral cartilaginous bar and the median portion of the connective-tissue wall of the caudal heart, while dorsad and ventrad it gradually blends in with the adjacent mesenchyme.

Now the question might be asked, What is the signifi-cance of this concentration of mesenchyme, and what will it form? Since cell-division is not particularly active here, it is quite certain that this mesenchyme has collected here, and is migrating inward to form the median ventral cartila-ginous bar and the connective-tissue layer of the heart. Moreover, from what we know about the manner of forma-tion of skeletal muscle in general, and the fact that no sharp line of separation can be determined between this mass of mesenchyme and the myotomes, together with the additional fact that the two are innervated by the same nerve, makes it reasonable to suppose that within this mass of mesen-chyme there are certain primitive muscle plate cells, that migrated inward along with the mesenchyme, but which, although at present indistinguishable from the mesenchymal cells, have preserved their identity, and will later multiply, arrange themselves outside the heart, and differentiate into m. cordis caudalis myoblasts. A point favouring the presence of early myoblasts in the m. cordis caudalis region is shown by the fact that the third ventral spinal nerve in the heart region, the one which innervates this muscle in the adult, has in the embryo (Fig. 11, *V. Sp. N.*) extended to the

immediate region of this muscle; while the preceding
ventral spinal nerve (Fig. 19, *V. Sp. N.*) has not reached a
level nearly as far ventrad.

In the 27 mm. series (Fig. 20) several important changes have
occurred in the region of the future m. cordis caudalis. The
former reticular appearance of the mesenchyme has to a large
extent disappeared, and in place of it, bunches of embryonic
white fibres have appeared everywhere about and between
the cells; the ventral boundaries of the myotomes (*myo.*)
have descended considerably ventrad, and in place of one
solid mass of concentrated mesenchyme plus a few hypo-
thetical undifferentiated myoblasts, as was described for the
20 mm. series as lying between the myotomes and the caudal
heart, we have in the 27 mm. series (Fig. 20), and more
strongly marked out in sections further cephalad, two quite
distinct areas. First a dense area situated directly laterad of
the caudal heart is composed of a great many round or
slightly spindle-shaped cells separated by a small amount of
embryonic white fibres, and a second thinner area, located
between the first area and the myotomes, consists of but few
cells and more embryonic fibres. The first area will un-
doubtedly form the lateral connective-tissue layer of the
caudal heart and the m. cordis caudalis; although in this
series the myoblasts cannot be detected from the embryonic
connective-tissue cells, still the general shape of the area
itself in transverse section resembles that of the m. cordis
caudalis of a later stage. The second or thinner area repre-
sents nothing more than mesenchyme changing into white
fibrous tissue.

Between the 27 mm. and the 58 or 60 mm. series there is a
considerable gap, resulting naturally in a marked progress in
the histogenesis of the two areas described in the previous
paragraph. The first area has differentiated into the lateral
connective-tissue wall of the caudal heart (Fig. 16, *F. C. T.*)
and into the m. cordis caudalis (Fig. 16, *m. c. c.*); while the
second area is now a mass of embryonic white fibrous tissue,
connecting the m. cordis caudalis with the myotomes. A

closer examination of the m. cordis caudalis will reveal it to be still in a very embryonic condition. Syncytii have formed of the myoblasts, and a few fibrillæ (Fig. 23, *Fib.*) are present; the general state of the muscle is about identical to the myotomes of the 20 mm. series. On the other hand the myotomes of the 60 mm. series have developed well-formed muscle-fibres (Fig. 23, *Myo. F.*).

In the 85 mm. series the first trace of muscle-fibres (Fig. 24, *M. C. F. F.*) have appeared in the m. cordis caudalis. They are still very small, and must increase considerably in diameter before adult conditions are reached. It may be said that in every way the m. cordis caudalis muscle-fibres resemble skeletal muscle rather than cardiac muscle. Turning to the myotomes for comparison we find that the muscle-fibres (Fig. 24, *Myo. F.*) have about reached adult conditions as regards size, but their fibrillæ (*Fib.*) are large and still few in numbers, and must subdivide considerably before an adult state is reached.

The m. cordis caudalis might be said then to have its origin in a very early stage (20 mm.) from myoblasts situated in a condensed mass of mesenchyme that is located between the base of the embryonic myotomes on one side and the embryonic caudal heart and the median ventral cartilaginous bar on the other side. For the most part this mass of cells is migrating mesenchyme that is moving mesad to form the connective-tissue layer for the caudal heart and the median ventral cartilaginous bar, but from our knowledge of the histogenesis of skeletal muscle, which this is, and the fact that it is innervated by the same ventral spinal nerve that innervates the myotomes, and that this nerve reaches the region of the m. cordis caudalis before the myoblasts are differentiated from the adjacent mesenchyme, leads one to believe that certain muscle plate cells must also have migrated inward along with the mesenchyme, and existed for some time in the above-described concentrated mesenchyme, and while they are indistinguishable from the surrounding mesenchymal cells, yet they would have retained

their identity, and eventually will develop into m. cordis myoblasts. The first appearance of the m. cordis caudalis occurs much later than the myotomes, and its fibres never reach anything like the size of the myotome fibres. Figs. 21 to 25 are intended to show the comparative stages of histogenesis of these two muscles from 20 mm. to 20 cm.

VIII. Summary and General Discussion.

Polistotrema in the adult possesses a distinct system of lymphatics or veno-lymphatics, supplying a region amply furnished with veins. No capillaries were observed connecting these vessels with the arteries, but they always contained some red corpuscles, usually great quantities in embryos, and only a few in the adult. In all of the larger and medium-sized embryos the connective tissue outside these vessels was so filled with red corpuscles as to resemble germinative centres, and the vessels themselves appeared to be reservoirs for storing them. On account of the loose state of the endothelium of these vessels it would be an easy matter for an external corpuscle to migrate through the wall, and in this instance it would seem to be a more plausible explanation for the appearance of red corpuscles in the veno-lymphatics than to regard them as extravasations from the blood-vessels caused by the rupturing of a delicate wall separating them. All of the subcutaneous canals are decidedly sinus-like, distinctly recalling the conditions found in the Amphibians. Posteriorly the entire system culminates in two pulsating hearts, which are mere enlargements of the two terminal forks of the caudal vein.

So far as is known, Polistotrema, Myxine, and the eels are the only fish-like vertebrates that possess pulsating caudal hearts. They swim by a snake-like movement, while most fish swim by rapid lateral vibrations of the caudal fin, and would, therefore, not require any specialized pulsating heart, for the motion of the fin against the wall of water would alternately press these caudal sinuses against the

hypural bones and the tail muscles, thus discharging their contents into the caudal veins and functioning as the caudal hearts of Polistotrema.

With the exception of some additions noted in connection with the hæmal lymphatics, the adult veno-lymphatics in the tail region were found to be about the same as Greene and Favaro had described them.

In my youngest stage the arterial system was well formed, while the venous system was in a rather late embryonic state. In the region of the post-gut the caudal vein was in communication with the yolk-sac veins, and near the tip of the notochord a branch on either side anastomosed with a corresponding branch of the caudal artery. Throughout the vein there was everywhere the remains of mesenchymal cavity partitions that had not fully disintegrated at this stage. The caudal vein was described as growing in diameter by the addition to either side of certain isolated mesenchymal spaces, but strange to say, the intersegmental veins were formed as dorsal and ventral sprouts from the lateral surface of the caudal vein.

While the early stages of the formation of the blood-vessels in Polistotrema were not to be had, considerable evidence was gathered in support of a hypothesis that I would like to propose for the origin of the blood-vessels, and which may also hold good for the lymphatics, namely, that the larger longitudinal trunks which follow the main axis of the embryo are developed through the formation and coalescence of certain mesenchymal cavities, while the intersegmental and smaller branches arise as outgrowths from the wall of the main trunks already formed. The process involved in connection with the larger trunks is identical to Lancaster's description of the formation of the blood-vessels in the leach by a vacuolation of the mesenchyme; some of the cells by flattening are going to form the endothelium, and others, by becoming round and increasing in size, are transformed into red corpuscles. In connection with the outgrowths of the intersegmental vessels from the walls of the main trunks it should be noted that all

observations on growing blood-vessels have been confined
solely to vessels of this character and not to the longitudinal
trunks.   The above hypothesis would be somewhat comparable
to the development of the nervous system, where you would
have the central nervous system being formed from one
primary layer of the embryo, and the nerves, which would be
analogous to the intersegmental vessels, arising as offshoots
from the main stem.

The rate of development of the caudal heart is rather slow :
it begins in embryos between 15 and 20 mm. and is taking
place in embryos of 85 mm. This construction stage doubtless
occupies a period of several months.

The caudal heart of Polistotrema differs from the lymph-
sacs of birds and mammals in that it never loses its primary
connection with the vein and acquires a second communication.
In the adult it is simply an expansion of the vein.

Concerning the construction of the caudal hearts of Polis-
totrema, they have been described as having been excavated
out of the mesenchyme dorsad and ventrad of the anterior
ends of the two forks of the caudal vein, through the disinte-
gration of the walls of large mesenchymal cavities, which for
the most part are connected with each other and the vein,
and which were originally formed from the isolated mesen-
chymal spaces after the manner set forth on p. 338.   The
process involved in the formation of these isolated mesenchymal
spaces is identical to what Lancaster found in the leach, namely,
a vacuolation of the mesenchyme by the disintegration of
certain of the mesenchymal cell processes, and the flattening
of some mesenchymal cells to become endothelium and a
rounding of others to become red corpuscles.

In connection with the 20 mm. series A, the possibility was
noted of the proximal ends of three so-called dorsal embryonic
intersegmental veins or capillaries contributing in a very
limited extent to the formation of the caudal hearts.

It will be seen, then, that the development of the caudal
hearts in Polistotrema as I have interpreted them is in
harmony with what Stromsten found in the turtle, but at

variance with a considerable recent work on the development of the lymph-sacs in the higher vertebrata.

Closely associated with the early construction of the caudal hearts occurs the formation of the ventral trunk, at first paired, and each consisting of a longitudinal chain of isolated mesenchymal cavities, which later become connected with each other and the caudal heart.

The paired valves guarding the orifices of the anterior and posterior communications between the ventral veno-lymphatic trunk and the caudal heart take their origin from a migration inward and dorsad of the mesenchymal wall on either side of the orifice.

The endothelium has already been described as coming from the flattening of some of the border-cells of the larger mesenchymal cavities, and in like manner the connective-tissue endocardium comes from a concentration and differentiation of the mesenchyme about the endothelial lining.

There is no true myocardium in the caudal heart of Polistotrema, but the functional myocardium, the M. cordis caudalis, arises from myoblasts in the centre of a mass of concentrated mesenchyme situated between the base of the myotomes and the caudal heart that is migrating inward to form the median ventral cartilaginous bar and the connective-tissue layer of the caudal heart. Since the muscle-fibres of the M. cordis caudalis are true skeletal muscle, their myoblasts are supposed to have originated from primitive muscle plate cells that have migrated inward with the surrounding mesenchyme. It was demonstrated that the ventral spinal nerve which innervated this muscle also supplied the myotomes, and in the early embryos reached the area of the future M. cordis caudalis long before its myoblasts were distinguishable from the adjacent mesenchyme.

In conclusion it might be said that my studies thus far indicate that the most primitive form of a lymphatic system are veins that function for both lymphatics and veins. Hence it would be expected that ontogeny would repeat the phylogeny of the lymphatics, and instead of having their

origin directly from veins, that they would begin exactly as the
veins did, namely, by the vacuolation of the original mesen-
chyme. In one of the primitive vertebrates, Polistotrema,
I have described these vessels as veno-lymphatics, by which
is meant a system of vessels that is more closely related to
the veins than the lymphatics of the higher vertebrata are. In
a later communication I expect to show that the main factor
in the construction of the veno-lymphatic system is the same
as was described for the caudal hearts, namely, the formation
and union of certain mesenchymal spaces.

The following paper was completed August 28th, 1912, and a
considerable of the later work on it was done at the Institute
of Anatomy of the University of Minnesota.

IX. EXPLANATION OF PLATES 19–21,

Illustrating Mr. William F. Allen's paper on " Studies on the
Development of the Veno-Lymphatics in the Tail Region
of Polistotrema (Bdellostoma) Stouti. First
Communication : Formation of the Caudal Hearts."

[With the exception of Figs. 4 and 4 A, which are from dissections of
an adult and a diagrammatic reconstruction, all figures are from trans-
verse sections or from accurate graphic reconstructions.

In the transverse sections all vessels were drawn in outline as tubes,
primary mesenchyme as cells with processes or as simple dots in the
low power drawings. A concentration or centralisation of mesenchyme
to form an organ is set forth by an increased number of these cells or
dots. A differentiation of mesenchyme into connective tissue is shown
by short irregular lines running in all directions. Muscle, when seen
by low power, is indicated by fine dots, and cartilage by much coarser
dots. All of the outlines, including the cells and their protoplasmic
processes, were made with the aid of a camera lucida.

Concerning the graphic reconstructions, most accurate drawings
were made of each, or part of each section, with a magnification of
100, 200, or 400 diameters. In case of the low-power reconstruction
drawings, either additional high-power drawings were made, or careful
comparisons were made with the high power, of each structure drawn.
At the outset, a base line from which all measurements were taken
was established by drawing a line at the level of the ventral surface

of the notochord at right angles to a median sagittal plane, and this base-line was added to each successive drawing by accurately placing the second or following drawing over the first above a plate of glass illuminated from below. In every reconstruction this base line is indicated by the dotted line (*B. L.*), and from it all the vessels and organs were measured off with dividers and plotted on ruled mm. paper.

The caudal artery in all the reconstructions is represented by circular cross-lines, the spinal cord by oblique cross-lines, the caudal vein and caudal heart by outlines only; the notochord, when completely reconstructed, by coarse dots, otherwise simply as a line to indicate its lower border. Certain mesenchymal spaces that are taking part in the formation of the caudal heart are indicated in outline. Of these there are two kinds: those indicated by small (*c.*) have not at this stage attained connection with the caudal vein; and another class, indicated by capital (*C.*), that have gained one or more connections with the caudal vein. When these cavities are located above or below or in front (laterad) of the caudal vein or other organ they are indicated in outline, but when they lie median to them they are indicated in dotted outline. Also any vessel lying median to an organ or other vessel is shown in dotted lines. A layer of mesenchyme separating the caudal vein from a mesenchymal space or two caudal heart mesenchymal spaces, or the caudal heart from the ventral veno-lymphatic trunk, is represented by a solid black space.]

LIST OF ABBREVIATIONS USED IN THE FIGURES.

A. or *P.* prefixed to an abbreviation signifies anterior or posterior, and *R.* or *L.* right or left. Embryonic structures are indicated by small letters, and adult or nearly adult by capital letters.

a. In reconstructions, marks the place of bifurcation of the caudal artery. *A. L. Hæ. T.* (1). Anterior fork of the posterior longitudinal hæmal lymphatic trunk. *b.* In reconstructions, marks the place of union of the two caudal veins. *B. L.* In reconstructions, base line from which all measurements were made. *B. V.* Blood-vessel. *C.* Caudal heart mesenchymal space that had assumed connection with the caudal vein or with other caudal heart mesenchymal spaces. *c.* Primitive caudal heart mesenchymal space that had not at this stage assumed connection with the caudal vein or with other caudal heart mesenchymal spaces. *Cau. A.* Caudal artery. *Cau. V.* Caudal vein. *C. T.* Connective tissue. *c. t.* Embryonic connective tissue. *d.* In Fig. 4 A, large veno-lymphatic sinus lying on the lateral surface of the last two mucous sacs. *D. A.* Dorsal fin-ray artery. *D. Bar.* Median dorsal

cartilaginous bar or basal fusion of the dorsal fin-rays or radials. *d. bar.* Massing of the mesenchyme to form the median dorsal bar. *Dep.* In Fig. 7 certain depressions in the lateral wall of the caudal vein, which doubtless represents the remains of an earlier embryonic condition of the vein. *D. R.* Dorsal fin-radials or rays. *D. S.* Dorsal fin-ray veno-lymphatic canals or sinuses. *D. V.* Dorsal veno-lymphatic trunk. *d. v.* Above in embryonic condition. *Ep.* Epidermis. *e. per.* Embryonic external perimysium. *F. C. T.* Fibrous connective tissue. *Fib.* Muscle-fibrillæ. *Hæ. T.* Intersegmental or hæmal lymphatic canal. *Ints. A.* Intersegmental artery. *Ints. V.* Intersegmental vein. *i. per.* Embryonic internal perimysium. *L. Cau. A.* Left caudal artery. *L. Cau. H.* Left caudal heart. *l. cau. h.* Above in embryonic condition. *L. Cau. H.* (1). Anterior portion of the left caudal heart. *L. Cau. V.* Left caudal vein. *L. Hæ. T.* Longitudinal hæmal lymphatic trunk or trunks. *L. Hæ. T.* (1). Posterior portion of the longitudinal hæmal lymphatic trunk. *L. L. Hæ. T.* Left longitudinal hæmal lymphatic trunk. *L. L. Hæ. T.* (1). Left branch of the posterior portion of the longitudinal hæmal lymphatic trunk. *L. L. S.* Left lateral veno-lymphatic sinus or trunk. *l. l. s.* Above in embryonic condition. *L. L. S. C.* Left lateral sinus mesenchymal cavity. *L. L. S. Con.* Left lateral sinus connection with the ventral trunk. *L. Proc.* Lateral process of the median ventral cartilaginous bar. *l. proc.* Above in embryonic condition. *L. V. V.* Left ventral veno-lymphatic trunk. Posterior portion homologous to caudal trunk of the higher fishes. *l. v. v.* Above in embryonic condition. *M. C. C.* Musculi cordis caudalis or myocardium of Favaro. *m. c. c.* Above in embryonic condition. *M. C. C. F.* Musculis cordis caudalis fibre. *M. C. C. Myob.* Musculi cordis caudalis myoblast. *Mes.* Mesenchyme. *M. Sac.* Mucous sac. *My.* Myelon or spinal cord. *Myo.* Myotomes. *myo.* Above in the process of formation. *Myob.* Myoblasts. *Myo. F.* Myotome muscle fibre. *Myo. Myob.* Myotome myoblast. *Nc.* Notochord. *O.* (1). First or anterior orifice between the ventral veno-lymphatic trunk and the caudal heart. *o.* (1). Probable point where the anterior orifice between the ventral trunk and the caudal heart will later occur. *O.* (2). Second or posterior communication between the ventral veno-lymphatic trunk and the caudal heart. *o.* (2). Probable place of the posterior communication between the ventral trunk and the caudal heart. *P.* In reconstructions, partition of mesenchyme separating two parts of the caudal vein or heart. Possibly the remains of a wall separating two cavities. *R. C.* Red corpuscle. *R. Cau. A.* Right caudal artery. *R. Cau. H.* Right caudal heart. *r. cau. h.* Above in embryonic condition. *R. Cau. V.* Right caudal vein. *R. L. Hæ. T.* Right longitudinal hæmal lymphatic trunk. *R. L. Hæ. T.* (2). Right branch of the posterior portion of the longitudinal hæmal lymphatic trunk. *R. L. S.* Right

lateral veno-lymphatic sinus or trunk. *r. l. s.* Above in embryonic condition. *R. V. V.* Right ventral veno-lymphatic trunk. Posterior portion homologous to the caudal trunk of the higher fishes. *r. v. v.* Above in embryonic condition. *S. Gan.* Spinal ganglion. *V. A.* Ventral or anal fin-ray artery. *Val.* (1). Valves guarding the anterior orifice between the ventral veno-lymphatic trunk and the caudal heart. *val.* (1). Above in the process of formation. *V. Bar.* Median ventral cartilaginous bar or basal fusion of the ventral or anal fin-radials. *v. bar.* Above in the process of formation. *Val. F.* Valve fold·extending up into the caudal heart from the sides of the orifice between the heart and the ventral trunk. *V. L. Hæ. T.* Ventral or inferior longitudinal hæmal lymphatic trunk. *V. R.* Ventral or anal fin-radials. *v. r.* Above in the process of formation. *V. S.* Ventral or anal fin veno-lymphatic canals or sinuses. *v. s.* Above in embryonic condition. *V. Sp. N.* Ventral spinal nerve ramus. *V. V.* Ventral veno-lymphatic trunk. Posterior portion homologous to the caudal trunk of the higher fishes. *v. v.* Above in the process of formation. *Z.* Indicates posterior ending of the embryonic caudal heart in reconstructions as located by the crossing of the third spinal nerve in the heart region.

Fig. 1.—Represents a transverse section through the tail region of an 85 mm. Polistotrema embryo; cut passing through the anterior region of the caudal hearts and viewed from the rear. This section shows the enormous lateral veno-lymphatic sinuses or trunks together with their dorsal and anal fin branches, the longitudinal dorsal and ventral veno-lymphatic trunks, and the caudal hearts in transverse section. Observe the left lateral sinus emptying into the left ventral trunk, and the right ventral trunk communicating with the right caudal heart; its orifice being guarded by two valves opening into the heart. It should be mentioned that the original section showed the great lateral veno-lymphatic sinus to be completely distended by blood-corpuscles. × 25.

Fig. 2.—Is from the same series as Fig. 1, but 390 microns cephalad. Less than one half of the section is figured. This section passes through the caudal hearts as they are about to terminate in the caudal vein. Note especially that the longitudinal hæmal lymphatic trunks have united to form a single posterior transverse stem, which passes ventrad and divides into anterior and right and left branches that empty separately into the ventral veno-lymphatic trunk after encircling the median ventral bar. × 25.

Fig. 3.—From same series as above. Section is taken several slides cephalad of Fig. 2, and only the main longitudinal hæmal vessels are shown in relation to each other and to the notochord. Observe the tendency for the longitudinal hæmal lymphatic trunks to divide into two

portions and for the ventral portion to receive an intersegmental branch. × 25.

Fig. 4.—Is from a dissection of the left caudal heart and the adjacent lymphatic and blood-vessels, excepting the lateral veno-lymphatic sinus and its dorsal and ventral or anal fin branches of an adult Polistotrema are omitted. Any attempt to represent the enormous lateral sinus in this drawing would necessarily obliterate everything that was more deep-seated. It should, however, be stated that both the dorsal and ventral canals had frequent communications with the lateral sinuses. × 2.

Fig. 4 A.—Diagrammatic reconstruction of the hæmal lymphatic system of a 20 cm. Polistotrema in the region directly cephalad of the caudal hearts as seen from the left side.

Fig. 5.—Transverse section through the tail of a 20 mm. Polisto-trema embryo, series B. Section passes through the anterior end of the caudal hearts, which are in the process of formation. Primarily it is intended to show the position of the embryonic caudal hearts and their relation to other developing structures, especially to the embryonic lateral sinus, which at this stage consists of several mesenchymal spaces uniting in front and behind, but which in a later stage will be one continuous and much larger cavity. At this stage none of the veno-lymphatic trunks have gained connection with each other or with the caudal heart. Note the large mesenchymal cavity (C.), which in the next section caudad (Fig. 13) connects with the caudal vein. Also a more ventral cavity (l. v. v.) is shown that has at this stage no connection with the caudal vein, and which for reasons stated in the text I take to be the beginning of the posterior portion of the ventral veno-lymphatic trunk. × 50.

Fig. 6.—Graphic reconstruction of the left caudal artery, left caudal vein, left embryonic caudal heart, and the left embryonic ventral veno-lymphatic trunk of a 20 mm. Polistotrema embryo, series B, as seen from the left side. Near the tip of the spinal cord a space of 640 microns has been left out of the reconstruction. Behind this the posterior ending of the notochord, spinal cord, and the union of the left caudal vein in the left caudal artery is clearly shown. Of especial significance are the mesenchymal spaces, (c.) and (C.), which are undoubtedly the main factors in the formation of the caudal hearts. × 200.

Fig. 7.—Identical reconstruction of the same structures of another 20 mm. Polistotrema embryo, series A, as Fig. 6, except that this reconstruction is not carried as far caudad. It should be noted in the caudal heart region that there is a direct anastomosis of an interseg-mental vein with an intersegmental artery. If the ventral veno-lymphatic trunk is present it is represented by a small mesenchymal

space (*l. v. v.*). In some respects series A is more embryonic than series B. × 200.

Fig. 8.—Graphic reconstruction of the left caudal artery, vein, heart, and the ventral and hæmal veno-lymphatic systems of a 60 mm. Polistotrema. Posteriorly the caudal heart is shown to be still in the process of formation, but in the main it has assumed adult conditions. Likewise the ventral veno-lymphatic trunk has attained practically adult conditions. Two connections are established with the left caudal heart, each of which is guarded by a pair of lateral valves, and since this is a median reconstruction, it passes between them, so that they are not shown in this figure. In the region immediately in front of the caudal hearts the longitudinal hæmal lymphatic trunks proper are practically the same as in the adult, but no trace of the ventral longitudinal hæmal lymphatic trunk has apppeared in this stage. × 50.

Fig. 9.—Graphic reconstruction of a portion of the notochord, the caudal artery and the left caudal artery, the caudal vein and the left caudal vein, the embryonic caudal heart and the embryonic ventral veno-lymphatic trunk of a 25 mm. Polistotrema. It should be noted that the caudal heart is fairly well formed anteriorly, but posteriorly it is in an early stage of formation, showing very conclusively that it is a gradual process, taking place from cephalad to caudad. This process is discussed at length in the text. Considerable progress is also shown in connection with the development of the ventral veno-lymphatic trunk over the 20 mm. reconstructions. An identical mesenchymal cavity (*l. v. v.* (1)) is shown, but it has increased in size and obtained connection with the embryonic caudal heart. Also additional mesenchymal spaces have put in their appearance in a direct line posterior to this, and in one place (*o.* (2)) there is a collection of small cavities, indicating possibly the place where the second or posterior connection with the caudal heart will occur. × 100.

Fig. 10.—Identical reconstruction of the same structures of a 27 mm. Polistotrema as the previous reconstruction of the 25 mm. Polistotrema. It, however, shows considerable advancement, although from its centre to its posterior border it is in more or less embryonic condition. Two large mesenchymal cavities (*C.*) that have gained connection with the caudal heart and vein are very conspicuous. From a lateral view the posterior might readily be taken for an intersegmental vein, or a degenerate intersegmental vein that was taking part in the formation of the caudal heart, but, as was shown in the text, its extreme median position is against this hypothesis. Note the marked and regular progress in the formation of the ventral veno-lymphatic trunk. Cavities (*l. v. v.* (1), (2)) have coalesced in one long cavity and cavities (*l. v. v.* (3), (4)) have also united, and both of these large cavities have

attained connection with the caudal heart. In two places they have gained connection with a mesenchymal space belonging to the embryonic lateral sinus, and in one place a direct connection has been established with the left lateral sinus. × 100.

Fig. 11.—Transverse section through the extreme posterior portion of the left embryonic caudal heart of a 20 mm. Polistotrema, series B. Its exact position is shown by the line 11 in Fig. 6. Note that the endothelium of the caudal vein is but little differentiated, and the cells and their processes cannot be told from the mesenchymal cells immediately outside that will be concerned in the formation of connective tissue and the median cartilaginous bar. Of especial interest is the primitive mesenchymal space (c.), which has not at this stage gained connection with the caudal vein, but which in a later stage would doubtless take part in the formation of the caudal heart. × 225.

Fig. 12.—Transverse section through the median portion of the embryonic caudal hearts of the same 20 mm. embryo as Fig. 11, but taken 200 microns cephalad; the exact position of the section is shown by the line 12 in Fig. 6. In the left heart note the large ventral and the small dorsal mesenchymal spaces, which have attained connection with the left caudal vein. Also the posterior tip of another cavity is seen laterad of the dorsal cavity, which expands further caudad and terminates in the left caudal vein. The embryonic right caudal heart consists of several ventral cavities (C.), which unite further caudad and cephalad in larger cavities that terminate in the vein. Also where the red corpuscle (R. C.) is suspended in the mesenchyme there is evidence of the beginning of a primitive mesenchymal space. In short, this section shows most all of the various stages of the mesenchymal spaces that go to make up the caudal heart. At every point the endothelium gives evidence of being differentiated mesenchyme, and the termination of the mesenchymal spaces in the caudal vein have the appearance of acquiring secondary connections with it rather than out-budding from it. × 225.

Fig. 13.—Transverse section through the extreme anterior end of the left embryonic caudal heart of the same embryo as Figs. 11 and 12, being taken 120 microns cephalad of Fig. 12, and its exact position in Fig. 6 is shown by the line 13. Observe that the orifice of cavity (C.) has the appearance of having acquired connection with the vein by the breaking down of the wall separating them. Also what I take to be the first mesenchymal space of the ventral veno-lymphatic trunk (l. v. v. (1)) is shown in cross-section. It is full of red corpuscles, and the loose mesenchyme above is possibly indicative of the place where the first or anterior communication with the caudal heart will occur. A comparison of the endothelium of the various regions with mesenchyme is of interest. × 225.

Fig. 14.—Transverse section through the centre of the left embryonic caudal heart of a 20 mm. Polistotrema, series A. Its exact position is shown by the line 14 in Fig. 7. Observe the direct anastomosis of a so-called intersegmental vein with an intersegmental artery and the termination of a mesenchymal space (C.) in the vein. This shows that an intersegmental vein may take some part in the formation of the caudal heart. A comparison of the adjacent structures is also of interest. × 130.

[Figs. 15 to 18 are introduced to show the nature of the orifice between the ventral veno-lymphatic trunk and the caudal heart, the mode of development of the valves guarding this orifice, and something of the structure of these vessels in the various stages.]

Fig. 15.—Transverse section through the anterior orifice between the embryonic ventral veno-lymphatic trunk and the left embryonic caudal heart of a 27 mm. Polistotrema. Note that the endothelium is barely differentiated from the mesesenchyme and that no valves have appeared, but that there is a proliferation of cells [val. (1)], which may be the forerunner of the valves. × 225.

Fig. 16.—Transverse section through the two valves guarding the first or anterior orifice between the ventral veno-lymphatic trunk and the left caudal heart of a 60 mm. Polistotrema. At a glance it will be seen that the valves have almost reached adult conditions, that they are formed by a proliferation and massing inward and dorsad of the mesenchyme between these two structures. At this stage the mesenchyme of the valves has not become differentiated into fibrous connective tissue lined with endothelium. These valves have made fully twice as much advance as the valves guarding the second or posterior orifice. It will be seen that the caudal heart is lined with endothelium, outside of which the mesenchyme has differentiated into a sort of fibrous connective tissue. The musculi cordis caudalis is well defined and fibrillæ are making their first appearance in its myoblasts. × 225.

Figs. 17 and 18 are transverse sections through the valves guarding the orifice of the ventral veno-lymphatic trunk with the right caudal heart of the same embryo as Fig. 16, and the two figures are from adjoining sections. Both show that the valves guarding this orifice are decidedly more embryonic than is the case with the opposite or left caudal heart-valves. The valves are here represented as two masses of mesenchyme that have grown inward and upward from the lateral sides of the orifice, having fused in some places and in others an open communication remains. In Fig. 17 the orifice is on the left side, in the next section cephalad (Fig. 18) there is no orifice, and in the next section cephalad of Fig. 18 the orifice is found to be on the right side. × 225.

[Figs. 19–25 have been introduced to show the comparative rate of growth and histogenesis of the myotomes and the musculi cordis caudalis.]

Fig. 19.—Transverse section through the lower portion of the embryonic myotomes and the embryonic musculi cordis caudalis of a 20 mm. Polistotrema, series A. In this stage the myoblasts in the ventral end of the myotomes are not as far advanced as they are further dorsad, having no sign of fibrillæ, and no sign of perimysium has thus far appeared. It is continuous, however, with a greatly thickened mass of mesenchyme, which has probably migrated inward to form the connective tissue for the caudal heart and other structures, and in this greatly thickened mass of mesenchyme there may be musculi cordis caudalis myoblasts (?) indistinguishable from the adjacent mesenchyme, and which may have migrated in from the mesoblastic somites with the mesenchyme. × 130.

Fig. 20.—From an identical transection to Fig. 19, but from a 27 mm. embryo, showing the same structures slightly advanced. Note the formation of the external perimysium and the separation of the myotomes from the thickened mass of mesenchyme described above, and that the protoplasmic processes of this mesenchyme has undergone a change possibly preparatory to the forming of fibrous connective-tissue fibres, while the m. cordis caudalis boundaries are outlined. × 130.

Fig. 21.—(1) On the left, a transection of a small portion of the embryonic myotomes taken from about the centre. (2) On the right, similar portion of the musculi cordis caudalis in transection. Note that the fibrillæ and internal perimysium have made their appearance in the myotomes, but not in the m. cordis caudalis. × 225.

Fig. 22.—Identical to Fig. 21, but from a 27 mm. series. External perimysium has appeared in the myotomes and a slight change has occurred in the intercellular spaces of the embryonic m. cordis caudalis. × 225.

Fig. 23.—Identical to Fig. 21, but from a 60 mm. embryo. The muscle-fibres of the myotomes are fairly well developed, and fibrillæ are making their first appearance in the myoblasts of the m. caudis caudalis. × 225.

Fig. 24.—Identical to Fig. 21, but from an 85 mm. embryo. The muscle-fibres of both the myotomes and the m. cordis caudalis are well-formed. Note the presence of Cohnheim's areas in the former and the much larger size of the fibres in the myotomes. × 225.

Fig. 25.—Identical to fig. 21, but from a small adult 10 cm. long. Both fibres have attained adult conditions. Note how much smaller the m. cordis caudalis fibre is than the myotome fibre. The m. cordis caudalis is also extremely rich in blood-vessels. × 225.

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CONTENTS OF No. 235.—New Series.

MEMOIRS:

On some Queensland Trematodes, with Anatomical Observations and Descriptions of New Species and Genera.[1]

By

S. J. Johnston, B.A., D.Sc.,

Demonstrator of Biology, University of Sydney.

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With Plates 22—27.

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THE Trematodes comprising the subject-matter of the present work were collected by officers of the Institute and forwarded to me from time to time by the director, Dr. Anton Breinl, together with notes on the location of the parasites in their hosts and on the methods of preservation employed. The specimens were fixed for the most part in hot 70 per cent. alcohol, or in sublimate acetic, hot or cold; and, in cases where the form of the worm seemed to require it, some were fixed under slight pressure. The specimens converted into whole mounts I have generally stained in Ehrlich's or Delafield's hæmatoxylin and cleared in clove oil; all the forms except two, which were represented by single specimens, were studied by means of serial sections as well as in whole mounts.

I wish to thank Dr. Breinl for giving me the opportunity to study these forms, some of which presented some very interesting features : I have also to thank Professor Haswell, in whose laboratories the work was carried out, for valuable suggestions and criticism, as well as for the loan of much of the literature from his private library.

The worms were obtained from mammals, birds, reptiles and fishes, and comprise the following species:

From MAMMALS :

  Opisthotrema cochleare Fischer, from the œsophagus of Halicore dugong.

  Rhabdiopœus taylori gen. et. sp. n., from the intestine of Halicore dugong.

From BIRDS :

  Echinostoma revolutum Froelich, from the intestine of Anas superciliosa.

  Patagifer bilobus Rud., from the intestine of Platalea regia.

  Typhlocœlum reticulare sp. n.,[1] from the intestine of Anseranas semipalmatus.

  Allopyge antigones gen. et. sp. n.,[1] from the intestine of Antigone australasiana.

From REPTILES :

  Polyangium linguatula Lss., from the intestine of Chelone midas.

  Octangium sagitta Lss., from the intestine of Chelone midas.

  Microscaphidium aberrans Lss.,[2] from the intestine of Chelone midas.

  Diaschistorchis pandus Brn., gen. n., from the intestine of Chelone midas and Chelone imbricata.

From TELEOST FISHES :

  Pleorchis oligorchis sp. n., from the intestine of Tetraodon hispidus.

  Steringotrema pulchrum sp. n., from the intestine of Tetraodon hispidus.

From ELASMOBRANCH FISHES :

  Petalodistomum polycladum gen. et. sp. n., from the body-cavity of Dasybatis kuhlii.

---

[1] As the relatives of these two forms usually occur in the body-cavity of birds, I made special inquiries about the location in the hosts, and am assured by the collector that they were obtained from the intestine.

[2] Collected by Dr. H. L. Kesteven at Mast Head Island.

Petalodistomum cymatodes sp. n., from the body-
cavity of Dasybatis kuhlii.

In the new species and genera a diagnosis of each is given,
together with a more extended account of the anatomy and
remarks on the affinities and position in the system.

FROM MAMMALS.

On two occasions a number of trematodes of remarkable
form were collected by Mr. F. H. Taylor, of the Institute,
from the intestine of dugongs (Halicore dugong), caught
off the Queensland coast. From the first animal examined
sixteen of these worms were obtained; from the second, ten.
As the discoverer of the form, I have associated Mr. Taylor's
name with the species. Allied in their general organisation
to the monostomid family Notocotylidæ, these worms exhibit
a number of peculiar and unique features, as set out in the
following description :

Rhabdiopœus[1] taylori gen. et sp. n. (Figs. 1–4 and 15–26.)

Diagnosis.—Large size, elongated, rounded at each end,
convex dorsally and concave ventrally. Ventral surface
covered with large hooks. Excretory pore on the dorsal
surface near the posterior end. Complex, protrusible
proboscis lies in a cavity near the posterior end of the
body. No pharynx; intestinal limbs joined at their posterior
ends by a transverse commissure. Genital pore close
alongside the sucker; copulatory organs very elon-
gated and comparatively narrow, the cirrus sac
surrounding only a part of the vesicula seminalis. Testes
symmetrically placed in the hinder end of the body, outside
the intestinal limbs. Ovary between the testes; large shell-
gland near the ovary. No Laurer's canal nor receptaculum
seminis. Yolk-glands behind the testes and outside the

[1] ράβδιον, a little rod; ποιειν, to make.

intestinal limbs. Coils of the uterus running transversely, very numerous and close together, extending outwards a little beyond the intestinal limbs. Eggs small, operculated, with a long filament at each end.

Host : Halicore dugong, in the intestine.

Type-specimen in the Museum of the Australian Institute of Tropical Medicine, Townsville, No. T. 33. Co-type in the Australian Museum, No. W. 363.

These worms are large in size, up to 22 mm. long by 5 mm. broad. In shape they are elongated and lancet-like, gradually tapering to a rounded point in front, broader and more bluntly rounded off behind. While the body is comparatively thin anteriorly, it becomes very thick at the posterior end, where the gonads and the peculiar digitate organ lie. The dorsal surface is convex; the ventral flat or slightly concave in front, but more deeply concave in the posterior region, where the lateral and posterior edges of the body are turned in ventrally.

The ventral surface of the body is covered by a dense mat of hooks, the bases of the thick shafts of which form a tessellated pattern all over it. Each hook, sickle-shaped in longitudinal section, consists of a stout shaft and a backwardly directed bifid point, turned almost at right-angles to the shaft. They are 0·107 mm. long and 0·064 mm. broad at the base. These hooks are set in a very thick cuticular layer (figs. 15 and 16), which is apt to peel off in large patches or to come off bodily from the whole surface. This may happen, not only in the case of specimens fixed in hot sublimate acetic, but also in those specimens fixed in hot 70 per cent. alcohol. The majority of the specimens, however, preserved this layer intact.

The sucker, almost circular in form, measures 0·733 mm. in diameter in specimens 22 mm. long—i. e. is almost one thirtieth the body-length. It is subterminal and directed ventrally. There is no pharynx, but the œsophagus, which is moderately long and stout-walled, leads directly out of the oral sucker. The intestinal limbs are very thin-walled. They

lie at first close together, but gradually diverge till they come
to occupy positions at the junctions of the middle with the
lateral thirds of the body, when they run backwards parallel
to the lateral body-edges till they reach the level of the testes.
Arrived at the anterior faces of the testes they bend inwards,
enclosing the ovary, but lying on the inner side of, and dorsal
to, the testes.   Between the posterior ends of the testes they
lie very close together.   Behind them they diverge in a
circular curve on each side, and, keeping pretty close to the
rounded posterior end of the body, become confluent in the
extreme posterior end.   In this circular part of their course
behind the testes a few short cæca are given off; otherwise
they are unbranched and run fairly straight.   They lie dorsal
to the loops of the uterus.

On the dorsal surface of the body, near the posterior end,
there is a large circular or oval opening leading into a spacious
chamber, in the floor of which the excretory pore lies.   Nine
tunnel-like, tubular spaces, arranged in an anterior and two
lateral groups of three each, branch off from this chamber
into the parenchyma of the body, which is here very thick,
and in each tunnel there lies a thick cylindrical finger-like
process.   Each lies quite free in its tunnel except at its base,
where it becomes continuous with the tissues of the body
(fig. 20).   The three in the anterior group are longer than
the others.   These processes are muscular and extensible,
and are capable of being thrust out for a considerable distance
through the opening on the surface of the body.   Each pro-
cess or proboscis is circular in transverse section (figs. 3 and
23) and possesses a strong musculature.   The circular fibres
form a complete layer on the surface.   Within this lies a
circle of strong longitudinal fibres.   Lying within the latter
are found glandular cells which form rhabdite-like bodies and
mucus or some similar homogeneous secretion.   The cavities
in which the processes lie are filled up, or partly filled up
with mucus, which contains myriads of small rod-like bodies
very similar to the rhabdites of Temnocephala and the
Turbellaria.   Each gland-cell is pear-shaped, and opens

on the surface of the process through a long duct which runs between the longitudinal muscle-fibres. Just at the tips of the processes the muscular layers are much reduced, and the gland-cells seem to have converted their entire protoplasm into mucus and rhabdites (fig. 4). Lying in the middle of the parenchyma of the process is a large space surrounded by a very definite muscular layer, as well as several smaller cavities. With the central space in each process a large branch given off from the excretory vesicle communicates (fig. 22). Fluid forced into this channel from the excretory system apparently takes part in the protrusion of the process, and, together with the action of the muscular system of the process, would render it tense.

These processes, according to Taylor, who found them, do not seem to be used in any way for attachment. The function of rhabdites in the flatworms seems to me to be obscure, no very satisfactory explanation having been offered for them. In this case they probably give a certain amount of stiffness to the secretion of the processes, and may have an irritating effect. The whole organ is most probably used for cleaning up an area of the wall of its host's intestine, so as to make a place to which the sucker can be effectively applied.

Muscular System.—The muscular layers of the body-wall are very strongly developed. A complete layer of circular fibres lies immediately adjacent to the cuticle ; within the circular is a thick layer of longitudinal fibres, and internal to this is a layer of diagonal fibres, somewhat thicker than the outer circular layer. A dorso-ventral system of large muscle-fibres is developed to a very marked extent (figs. 15 and 16). The majority of these are fairly perpendicular to the dorsal and ventral surfaces of the body; many, however, are oblique in their direction. At each end, at the point where they reach the level of the diagonal fibres of the body-wall, these dorso-ventral fibres become divided into a number of branches, so that the area of their insertion into the cuticle is very much greater than the area of the cross-section of the fibre.

Excretory System.—The large branched vesicle opens into the proboscis chamber. The pore is provided with a strongly developed sphincter which is capable of keeping it closed when the fluid of the vesicle is being forced into the finger-like processes of the proboscis. The vesicle divides into four wide bays anteriorly and each of these bays becomes gradually narrowed into one of the four main trunk vessels. Those placed laterally diverge as far as the testes, round the outer sides of which they skirt, and then run forwards parallel to the sides of the body. Towards the anterior end they gradually trend inwards, and at a level just in front of and ventral to the intestinal fork they become joined in the form of a circular arch (fig. 26). All along their course they are provided with a number of wide side branches, regularly given off on the outer side and extending to the edge of the body (fig. 1). These branches do not anastomose with one another. The two vessels given off from the vesicle nearer the middle line pass the testes on the inner side and run forwards parallel to and within the intestinal limbs. They also give off a number of branches, but end blindly in front near the intestinal fork, without forming a transverse anastomosis. The four main excretory vessels and the intestinal limbs are placed approximately in the same plane, so that they all appear together in the same horizontal (parallel to the ventral surface) section (fig. 26).

Nervous System.—A pair of large cerebral ganglia are connected by a very thick commissure which crosses dorsally over the middle of the œsophagus (fig. 19). The principal trunks, in the form of a pair of lateral nerve-cords, gradually diverge outwards towards the sides of the body, at the same time sinking ventrally so that at a point not far behind the intestinal fork they come to occupy a position immediately internal to the muscular layers of the ventral body-wall (fig. 15). From the lateral trunks many small nerves are given off and are distributed laterally, inwards and dorsally.

Reproductive Organs.—The genital opening is at the anterior end, on the ventral surface, quite close to the sucker

on the right side near its middle. The testes are very large, about 2 mm. by 0·94 mm., with deeply lobed outline, and lie just behind the junction of the third and the last body fourths. They lie side by side, one on each side of the middle line, with their long axes obliquely placed, so that while their posterior ends are near together their anterior ends are some distance apart. Between the testes, within the bay formed by their divergence, the ovary and shell-gland lie, near the middle line.

The two vasa deferentia, arising near the middle of the anterior surface of each testis, meet just in front of the ovary to enter the vesicula seminalis. The latter is of extraordinary length and pursues a tortuous course, never far from the middle longitudinal line of the body, through half the animal's length. It occupies a position dorsal to the coils of the uterus. Only a comparatively small part of it lies within the cirrus sac, which is a cylindrical, muscular tube (figs. 24 and 25) running pretty straight through the anterior fourth of the body. Arrived in the posterior end of the cirrus sac the vesicula seminalis soon gives place to the ejaculatory duct, traversing the muscular penis. The proximal end of this ejaculatory duct is surrounded by a conspicuous prostate (fig. 25), which causes the cirrus sac to be much wider behind than in front. The penis and sac are of very unusual length compared to their diameter.

The ovary is a smooth-edged, oval body of considerable size, 0·89 by 0·57 mm., though much smaller than the testes. The " shell-gland," which lies alongside, is also very large, 0·82 by 0·49 mm. The oviduct, after traversing this organ, begins a series of transverse windings that run on as the uterus, in the form of a tube of comparatively narrow bore, but very great length. The very numerous coils of the uterus lying close together run from side to side in the ventral part of the body. Situated ventral to the vesicula seminalis, intestinal limbs and main excretory vessels, they fill up all this part of the body from the gonads behind to the level of the meeting of the first and second body fourths. From this point the vagina runs

forwards in a fairly straight course, more or less parallel to
the cirrus sac, and about equal to it in length, to the genital
opening. The coils of the uterus in a lateral direction reach
the outer excretory trunks. There is no Laurer's canal nor
receptaculum seminis.

The yolk-glands are situated laterally behind the testes.
Each lateral mass consists of grape-like groups of oval
follicles, which are small oval bodies varying from 0·099 by
0·064 to 0·107 by 0·077 mm. There are 12 to 15 follicles in
each group; and each lateral mass consists of about 50 of
these groups.

The eggs are thin-shelled, 0·026 mm. long by 0·015 mm.
broad, operculated, and with a long filament at each end
(fig. 2, *a* and *b*). The filaments vary a good deal in length,
apparently becoming longer during their passage along the
uterus; for while those eggs in the first few coils of the
uterus, near the ovary, generally possessed quite short fila-
ments, all the eggs in the more distant coils had quite long
filaments, up to 0·279 mm. long, i. e. more than ten times
the length of the egg.

Rhabdiopœus appears to me to be a member of the
family Notocotylidæ Lühe and sub-family Notocotylinæ
Kossack (**18**), in spite of the absence of the rows of glands
on the ventral surface generally found in members of this
group, and in spite of the presence of the complex proboscis.
These two structural characters are special features of perhaps
generic rank, but not, in my opinion, of sufficient importance
to separate the form from Kossack's sub-family. In their
general organisation they agree well with the members of
that sub-family (**18**, p. 554), coming nearest perhaps to Cata-
tropis Odhner (**48**). The general arrangement of the repro-
ductive organs and their ducts agrees fairly well with what
obtains in Catatropis, e. g. the position and form of the
testes and ovary; the form and situation of the vesicula semi-
nalis, cirrus sac and cirrus, of the uterus and vagina; the
vitelline glands, however, differ in lying behind instead of
in front of the testes. The structure of the excretory system

also agrees pretty well with that of Catatropis. Rhabdio-
pœus differs from the latter further in having the intestinal
limbs fused behind, in the absence of the serial ventral
glands, as well as in the presence of the complicated proboscis
and the thick layer of spines on the ventral surface.

Opisthotrema cochleare Fisch.

Eleven specimens were found in the œsophagus of
Halicore dugong, by Dr. Strangman, at Port Darwin.
They were smaller than Semper's specimens, on which
Fischer worked, and were all sexually immature. The
largest specimen measured 7 mm. long by 4 mm. broad.
The majority were close to this in size, while the smallest
was only 3 mm. long. The sexual ducts were all well formed,
though the gonads themselves were only represented by very
small patches of cells. The already formed cirrus distinguishes
these specimens at once from O. pulmonale von Linstow
found in the lungs of the same host. The position of the
testes is also different, being external to the intestinal limbs
in O. cochleare, but internal to them in O. pulmonale
(**24**). Fischer (**14**) mentions the spines on the ventral surface
of the species under discussion, but makes no mention of the
fact, readily seen in sections, that the anterior part of the
dorsal surface bears smaller, more scattered spines.

From Birds.

Fam. Echinostomidæ.

Echinostoma revolutum Froel.

One complete specimen, and a fragment consisting of the
posterior half, were obtained from the intestine of the
"black-duck," Anas superciliosa. According to Looss
this species of worm (described under name E. echinatum
R.) exhibits considerable variation, and the description given

by him (**30,** pp. 680–684) fits this specimen well. It is
12 mm. long by 1·9 mm. wide. There are 37 wreath spines,
15 dorsal, 6 on each side, and 5 on each ventral lobe. In its
anterior part the integument of the body is thickly covered
with small spines, which gradually decrease in number
towards the posterior end, and quite vanish at the level of
the posterior testes. The tests are as broad as long, and
very deeply incised, so that each is six-lobed. I follow Dietz
(**13**) in placing it as E. revolutum Froel.

Patagifer bilobus R.

A single specimen of this species was obtained from the
intestine of the black-billed spoonbill, Platalea regia.
It was fixed in sublimate-acetic-alcohol, slightly flattened,
stained with hæmatoxylin, and mounted whole. It measures
17 mm. in length and 1·4 mm. in breadth anywhere behind
the ventral sucker, except near the posterior end, where it
narrows to a round blunt point; it is somewhat wider at the
level of the ventral sucker. Except that the lateral wreath
spines are pointed at their outer ends, it agrees in all respects
with the description given by Dietz (**13,** pp. 417–419), and by
Looss (**30,** p. 685), for P. bilobus from Platalea leuco-
rodia.

Fam. Monostomidæ.

Sub-fam. Cyclocœlinæ.

Typhlocœlum reticulare sp. n. (Figs. 5, 6, 7, 27–30, and 33.)

Diagnosis.—Small, flat, leaf-like worms, about 4 mm.
long. Integument smooth. Sucker very weak, with its
opening ventrally placed. Intestinal limbs with short cæca
on their inner sides; joined together by a transverse loop
at their posterior ends. Excretory pore on the dorsal surface
near the posterior end; vessels forming a ventral network.

Genital pore on the ventral aspect, just beneath the œso-phagus. Testes large, m u c h branched bodies; copulatory organs moderately developed. Ovary oval, small; no Laurer's canal n o r r e c e p t a c u l u m s e m i n i s. Uterus richly coiled i n d o r s o - v e n t r a l l o o p s, n o t p a s s i n g o u t w a r d s be-y o n d the intestinal limbs, and filling up all the space between the intestinal limbs from the gonads behind to the intestinal fork in front. Follicles of the yolk-glands minute (0·022 mm.), extending from the beginning of the intestine in front, form-ing a loop behind the intestinal commissure behind.

Eggs 0·107 by 0·073 mm. (?)

Host: The pied goose, A n s e r a n a s s e m i p a l m a t u s, in the intestine.

Type-specimen in the Museum of the Australian Institute of Tropical Medicine, Queensland, No. T. 34.

Co-type specimen in the Australian Museum, No. W. 365.

Five specimens were obtained from the intestine of the pied goose, A n s e r a n a s s e m i p a l m a t u s, at Townsville.

They are small leaf-like forms, oval in shape, quite flat, and thin dorsoventrally, 4·3 mm. long by 1·83 mm. broad. The integument is quite smooth.

The anterior sucker is so weakly developed as to be almost non-existent. It is represented by a funnel-shaped depression opening on the ventral surface near the anterior end; its walls are provided with a few weak muscle-fibres, which are more concentrated near its base. At the bottom it opens into a very well-developed and muscular pharynx, 0·228 mm. long by 0·163 mm. broad.

The œsophagus is short but distinct, and divides behind into the two intestinal limbs, which run backwards parallel to the edge of the body and fuse together so as to complete the ring at the posterior end. A few short cæca are given off on the inner aspect of each limb.

Excretory System.—The excretory pore is on the dorsal surface, near the posterior end. It is surrounded by a distinct sphincter, and leads into a more or less spacious chamber situated wholly behind the intestine (fig. 5). Into

its anterior end two main excretory vessels open. These cross over the posterior intestinal loop and run forwards as far as the sides of the pharynx, where they end blindly. In their forward course they lie close to, and fairly parallel to the intestinal limbs, re-crossing them near their anterior end. On their outer sides they give off a number of mostly un-divided branches; on their inner aspect they give off a number of branches which, anastomosing freely, form a network in the middle of the body (figs. 6 and 33) lying ventral to the uterus. From all the branches finer tubes are given off which end in flame-cells. These finer tubes are apparently intra-cellular, as not more than one nucleus could be made out in the wall of any of those examined. The walls of the larger vessels—the main trunks, network and branches— as well as the vesicle are composed of a nucleated syncytium.

Nervous System.—Two large cerebral ganglia, com-posed of the usual nerve-cells and fibres, lie, one on either side, in front of the pharynx (fig. 29). They are joined by a thick commissure, and give off a number of fine nerves to the integument in the front of the body. Behind, each gives off a thick nerve, the lateral nerve-cord, containing in its course many nerve-cells as well as fibres (fig. 7). These nerve-cords run backwards just outside the intestinal limbs, and in the extreme posterior end of the body, behind the excretory vesicle, join together to complete the circuit. Branches are given off, both on the outer and inner aspects of these nerve-cords, and at the points where they leave the main trunks little heaps of nerve-cells usually occur. The internal branches fuse with one another so as to form a network on the ventral surface of the body, just internal to the muscular layers, and ventral to the network of excretory vessels (fig. 27).

Genital System.—The common genital pore is situated on the ventral surface just beneath the œsophagus—i. e. in front of the intestinal fork. The testes are large branched bodies lying near the posterior end, but completely within the space bounded by the intestinal loop. They are somewhat obliquely placed one behind the other. The posterior, lying

in the bay of the posterior intestinal commissure, extends forward on the left side as far as the ovary in front. The anterior, while it does not extend so far posteriorly, reaches in front nearly to the middle of the body. The two vasa deferentia, each formed by the confluence of several smaller ducts, join one another about the middle of the body, whence they run forward as a single tube, ventral to the uterus, to a level approaching the anterior part of the intestine. Here this tube expands into an elongated vesicula seminalis which lies within the posterior end of the weakly developed cirrus sac. The latter lies partly in front of and partly behind the intestinal fork ending at the genital pore. The ejaculatory duct is somewhat twisted, and bears a small prostate in relation to its proximal end. The ovary is a small oval body (0·142 by 0·086 mm.), situated on the left side just inside the intestinal limb and in front of the posterior testis. The oviduct, running at first posteriorly, soon expands into the ootype, which is surrounded by the "shell"-glands and lies behind the ovary. The uterus, entirely confined to the space between the intestinal limbs and filling up almost the whole of this space, is closely coiled, and presents a peculiar appearance in whole mounts owing to the coils running backwards and forwards between the dorsal and ventral surfaces (figs. 28 and 30). The terminal part is a well-marked vagina opening into the genital chamber.

The yolk-glands, consisting of very small and very numerous follicles (·022 mm. in diameter), cover the whole of the outer aspect of the intestinal limbs, and to some extent spread right round them, but are mainly confined to their ventral and outer surfaces. The two lateral groups become continuous round the posterior aspect of the intestine. The transverse yolk-ducts leave the longitudinal ones about the level of the ootype, and join together to form a small yolk reservoir that opens into that chamber. There is no Laurer's canal nor receptaculum seminis.

In none of the specimens did the uterus, a comparatively immense tube, contain any eggs, but a few were found on the

surface of the body or in the intestine. As no other trema-
todes were found in the host, which was subjected to a careful
examination, and the form and size of the eggs found conform
pretty well with those in closely related species, it is almost
beyond doubt that these eggs belong to the animal with which
they were found associated. They are large, oval in shape,
yellow in colour, 0·107 mm. long by 0·073 mm. broad.

This worm appears to be closely related to Typhlocœlum
cucumerinum Rud. as described by Kossack (**18**, pp. 543–
548), in spite of the occurrence of the small ventral sucker in
the latter. I have been able to find no trace of such a sucker
in my specimens, either in the whole mounts or sections. The
occurrence of such a sucker in worms, in all other respects
obviously associated with the Monostomidæ, must, I think, be
looked upon as an atavism—a thing of local interest, but of no
great systematic importance.

T. reticulare differs from T. cucumerinum in size and
shape; in the more elongated form of its pharynx; in the
greater extension forward of the anterior testis and the greater
length of the separate vasa deferentia; in the arrangement of
the yolk-glands behind the intestine; in the much smaller
size of the ovary; in the very characteristic dorso-ventral
winding of the uterine loops; and in the considerably smaller
eggs.

Allopyge antigones gen. et sp. n. (Figs. 7, 31 and 32.)

Diagnosis.—Size large, body muscular, narrowed towards
the ends; small dorsal muscular plug projecting into the
cavity of the oral sucker. Intestinal limbs hardly branched, not
straight, but undulating. Genital pore at or behind the
intestinal fork; cirrus sac lying therefore entirely behind
the intestinal fork. Copulatory organs moderately developed.
Yolk-glands more or less in grape-like follicles, lying closely
upon the ventral and outer aspects of the intestinal limbs,
meeting behind the intestine and extending forwards nearly
up to the intestinal fork. Ovary and testes lying on a

straight line, inclined at an angle to the antero-posterior axis
of the body; but the ovary not so closely associated with the
posterior testis as in Hyptiasmus. Anterior testis separated
from the posterior and the ovary by a number of uterine
loops. Uterine loops, in the posterior half of the body, exten-
ding out beyond the intestinal limbs; but no loops exten-
ding backwards beyond the posterior testes as in Hypti-
asmus. No Laurer's canal; a receptaculum seminis
present or absent.

Type species: A. antigones; from the small intestine of
the Australian crane, Antigone australasiana.

Type-specimen in the Museum of the Australian Institute
of Tropical Medicine, No. T. 35.

Co-type in the Australian Museum, No. W. 366.

This genus differs from Hyptiasmus, to which it is closely
related, mainly, in the undulating course of the intestinal
limbs, in the position of the genital pore, at, or just behind,
the intestinal fork, and in the consequent situation of the cirrus
sac entirely behind it; in the absence of uterine loops passing
behind the posterior testis, and in the presence (probably
invariable) of a receptaculum seminis. With this genus
probably Hyptiasmus ominosus Koss. and H. adolphi
Stoss. should be associated. I have not been able to see the
original text of Stossich's work (57), but know its contents.
only through Braun's abstract in the 'Zoologisches Centralblatt'
and by means of Kossack's criticisms of it, so that I do not
know whether H. adolphi is the worm of this group in which
Stossich found the receptaculum seminis, but suspect that it
is so. Three specimens of A. antigones were obtained from
its host; one was sectioned, and the other two were made into
whole mounts. They are large, flat, leaf-like worms, about
20 mm. long and 4 mm. broad. While the ventral surface is
flat the dorsal is somewhat convex. The integument is rough
with little tranverse corrugations.

The character of the oral sucker and the pharynx is exactly
the same as in T. reticulare. The structure which I have
called the pharynx has been generally looked upon, in related

species, as the oral sucker. Van Beneden, for instance (**2**), calls it the " bulbe buccale "—terms he applies to the oral sucker in other malacocotyleans described by him. He noticed the funnel-shaped depression in the anterior end of the body leading down to this " bulbe buccale," and his fig. 4, pl. xii, in the work quoted corresponds closely to the condition of affairs in T. reticulare and A. antigones. The walls of this funnel-shaped depression, in the specimens examined by me, are provided with muscular fibres, especially at its base (fig. 31), and I am convinced that here we have to do with the real oral sucker, very poorly developed though it be.

Monticelli (**40**) has called it a præ-pharynx, and Kossack (**18**) a præ-pharynx (p. 501) and a mouth-cavity (" mundhöhle," p. 543). The pharynx is a typical malacocotylean pharynx not only in regard to its position and shape, the structure of its muscular walls and of its lining, but also in its relation to the nervous system, the two cerebral ganglia lying completely in front of it (fig. 29). This structure both Monticelli and Kossack recognise as the pharynx. The walls of both the oral sucker and the œsophagus are richly supplied with gland-cells.

In A. antigonis the pharynx measures 0·41 mm. long by 0·25 mm. wide, the œsophagus, 0·244 mm. long, the anterior testis, which is round, being 0·733 mm. in diameter, the posterior, which is oval and transversely placed, being 1·059 mm. long by 0·896 mm. broad. In outline the testes are slightly indented. The ovary, which is oval and smooth-edged, measures 0·407 by 0·326 mm.

The ventral network of excretory vessels present in T. reticulare and A. antigones probably also occurs in other related forms. Van Beneden (**2**, p. 72) speaks of an anastomosis near the anterior end between the two main trunks in Monostomum mutabile, and shows it in his figure (**2**, fig. 3), but obviously overlooked the ventral network through the want of a good series of sections.

In A. antigones the network is more richly developed, and there is a dorsal as well as a ventral network. The form

and position of the vesicle and its pore are much the same as in T. reticulare. The nervous system of the former also corresponds pretty closely with the arrangement described for the latter.

In the uterus the loops lying between the anterior testis and the middle of the body extend outwards beyond the intestine almost to the edge of the body, while the more anterior loops are confined to the space between the intestinal limbs. There are no backwardly directed loops extending behind the posterior testis as in Hyptiasmus. While there is no Laurer's canal, a very distinct receptaculum is present (fig. 32).

The eggs are very numerous, smaller and narrower than in T. reticulare, 0·094 mm. long by 0·055 mm. broad, yellow to light brown in colour.

From Reptiles.

Fam. Angiodictyidæ.

Polyangium linguatula Lss.

Ten specimens of this species were obtained from the intestine of Chelone midas.

Octangium sagitta Lss.

About one hundred specimens were obtained from Chelone midas, living along with the Polyangium.

Fam. Pronocephalidæ.

Diaschistorchis pandus Braun, gen. nov. (Figs. 9, 10, 34–37.)

In addition to a single specimen obtained from the intestine of a hawksbill turtle, Chelone imbricata, which I caught in Port Jackson, I have received from the Institute six specimens of a worm obtained from the intestine of Chelone midas, caught off the Queensland coast. These worms appear

to me to be identical with Braun's Monostomum pandum
(**9**, p. 48) on the following grounds :

Professor Braun had at his disposal only a single specimen,
which was mounted whole. As the worm is fairly thick and
dense, it is, of course, impossible to view its anatomy com-
pletely in a single specimen.

As far as Braun's description goes my whole mounts
agree with it very well, the differences being of such minor
importance as to make it out of the question, in my opinion,
to propose a separate species for my specimens. A study of
serial sections, however, not only makes clear important points
in the animal's structure that are quite impossible to see in
the whole mounts, but also shows some of the appearances
in the latter to be somewhat misleading. While the worm is
undoubtedly one of the Pronocephalidæ Lss. (**33**), it does
not fit into any of the genera at present established, so that I
am obliged to propose a new genus for it, adding yet another to
the list of genera containing only a single species in this family.

Four of the worms available to me for study I cut into
sections, one transverse, one sagittal, and two horizontal
longitudinal. With the help of these series I shall supple-
ment Braun's description (**9**, pp. 48–50), afterwards giving a
diagnosis of the genus Diaschistorchis, and a discussion
of its relationships.

External Characters.—My specimens were all a little
smaller than Braun's, varying in length from 8 to 9 mm.,
while being 2·5 mm. broad at the level of the cirrus sac and
3 mm. broad at the widest part, near the posterior end. The
form of body is skiff-like, rounded at both ends, rather
narrower in front, and gradually increasing in breadth to
near the posterior end. The collar is less conspicuous than
in the other members of the family, taking the form of a low
kidney-shaped elevation of the surface of the body round the
sucker on the dorsal side, but not produced into lobes or
processes of any kind at its ventro-lateral ends. The dorsal
surface of the worm is arched, both from side to side and, to
a certain extent, antero-posteriorly ; the ventral surface is

concave, and sometimes the posterior edge is turned forwards a little. The genital pore is found on the ventral surface, near the left side, some distance from the anterior end; the excretory pore is on the dorsal surface, a little in front of the posterior extremity. The sucker measures 0·57 mm. by 0·63 mm. in its longitudinal and transverse axes respectively in the smallest specimen, and 0·717 mm. by 0·782 mm. in the largest. Its opening is subterminal.

Alimentary Canal. — A thick - walled œsophagus, 0·326 mm. long, leads back from the sucker, and joins the intestine almost at right angles, the limbs running at first transversely and in the same straight line. There is no pharynx. The intestinal limbs extend backwards to the level of the excretory pore, and are provided with numerous side branches, both on their outer and mesial surfaces. Some of these lateral cæca become divided into two or three short branches (fig. 34). The intestinal limbs do not become arched in towards the median plane in the region of the testes, as so commonly happens in members of this family, but pursue a course fairly parallel to the sides of the body, converging a little at their posterior ends.

Excretory System.—The excretory vesicle is provided with the characteristic funnel-shaped end, bearing processes on its walls—the "rippen" of Looss (33)—as in Epibathra and other related genera. The capacious vesicle extends forwards as far as the ovary, and is produced on each side into three or four wide diverticula. From one of these pairs —the second from the front—the main vessels are given off and extend outwards till they reach the outer sides of the testes. Here each vessel divides into two branches, one running posteriorly, the other anteriorly (fig. 10). Each anterior branch pursues an undulating course, in seven or eight large waves, ending blindly near the sucker. Branches are given off both from the outer and inner aspects; the former end blindly, but the latter anastomose with one another in the space between the intestinal limbs, ventral to the uterus.

The Nervous System is of the usual type, consisting of a large cerebral ganglion on either side of the œsophagus, joined by a thick transverse commissure, and giving off a number of nerves besides the main, lateral nerve-cords. The latter run backwards in the interval between the intestinal limb and edge of the body on each side, giving off nerves to various parts (fig. 37).

Reproductive System.—In whole mounts the male and female ducts appear to open on the surface separately, but the sections show them opening into a shallow genital chamber (fig. 35), which lies laterally on the left side, outside the intestine. The testes are not only deeply lobed, but actually split up on each side into from four to six separate pieces, which meet in the middle line behind, so that the whole forms a U-shaped structure. The anterior pieces lie exactly ventral to the intestinal limbs; posteriorly they gradually converge on the middle line. The separate pieces are themselves deeply lobed. In whole mounts the separate parts, which are only connected by the ducts, overlap one another somewhat when viewed from the dorsal or ventral aspects, so that their separate identity is not quite apparent. The two vasa deferentia pursue a separate course forwards for some distance, and on meeting continue on as a single duct on the right side, between the uterus and the yolk-glands. The enlargement of this tube to form the vesicula seminalis occurs some distance behind the anterior end of the yolk-glands. The part of the vesicula seminalis lying in the region of the yolk-glands, the unpaired vas deferens and the separate right vas deferens are about equal in length, each lying near a third of the length of the yolk-glands. The vesicula seminalis is coiled throughout its whole length, the short part lying within the base of the cirrus sac, taking the form of an S-shaped curve.

The cirrus sac is a strongly developed, elongated, club-shaped structure, with thick muscular walls, lying obliquely across the body in the hinder part of the anterior body third. There is a well-developed pars prostatica, marked off from the

ejaculatory duct and penis by a shallow constriction. The vagina, which is surrounded near its termination by a mass of unicellular glands, is only about half as long as the cirrus sac.

The ovary is deeply lobed, about as large as one of the pieces of testis. It lies to the right of the middle line on a level with the anterior end of the testis. A large "shell-gland" lies to the side and somewhat behind it, in the middle line. Laurer's canal is present but there is no receptaculum seminis. The transverse yolk-ducts meet to form a small yolk reservoir on the dorsal surface of the "shell-gland." The small rounded follicles of the yolk-glands are massed in grape-like bunches, there being from eight to twelve of these bunches on either side. They lie on the ventral side of the intestine and extend from the testes behind to a level a little in front of the middle of the body. The coils of the uterus are confined to the space between the intestinal limbs.

The eggs measure 0·033 mm. long by 0·019 mm. broad. Those in the proximal part of the uterus have no filaments, but the older eggs in the more distals coils appear to possess a single fairly large filament at the narrow end attached to the operculum, and a bunch of fine filaments at the other end. In the sections a few of these eggs exhibited this character clearly, but in the majority of cases the filaments appear to have become separated and lie in the uterus amongst the eggs, in masses.

Diagnosis.—Body above middle size, skiff-like. Collar less conspicuous than in the other genera of this family, taking the form of a low swelling round the head. Intestinal limbs extending straight backwards as far as the excretory pore; not arched inwards towards the middle line in the region of the testes; richly provided with side branches throughout their whole length both on their inner and outer aspects. The excretory vesicle reaches the "shell-gland" in front, with wide lateral diverticula; the main branches running up to the head end in an undulating course, giving off branches, some of which anastomose. Genital

pore outside the intestinal limbs ; copulatory organs strongly developed ; cirrus sac large and club-like, vesicula seminalis long and coiled, extending back more than half-way to the gonads. Testes forming a ∪ in the posterior end of the body, deeply lobed, and split up into several separate pieces on each side. Ovary lobed, near the anterior end of the testes. Laurer's canal, but no receptaculum seminis. Eggs with filaments.

Diaschistorchis is distinguished from all the other genera of its family by the less conspicuous development of the collar, by the form of the testes and the more complex arrangement of its excretory vessels, besides differing from each genus in a number of other respects. In appears to be nearer to Epibathra and Glyphicephalus than the others, agreeing fairly well with these in the general configuration of their organs except the testes ; and, in the arrangement of its excretory vessels, differing from these less than from the other genera.

Specimens in the Museum of the Australian Institute of Tropical Medicine, No. T. 36.

From Fishes.

Fam. Fasciolidæ.

Pleorchis oligorchis sp. n.   (Figs. 11, 38–40.)

Diagnosis.—Above middle size, rectangular in shape, without spines. Oral sucker very large and strong; ratio of oral to ventral sucker 7 : 3. Pharynx a little smaller than the ventral sucker, short œsophagus, intestinal limbs wide and straight, with a single anterior cæcum on each side Excretory vesicle reaching the receptaculum seminis in front. Genital pore in middle line, in front of and very close to the opening of the ventral sucker. Testes in two parallel rows, generally five on one side and six on the other; no cirrus

sac. Ovary spherical; Laurer's canal and receptaculum present; uterus small; yolk-glands very richly developed.

Parasitic in the intestine of Tetraodon hispidus Linn. Type specimen in the Museum of the Australian Institute of Tropical Medicine, Townsville, No. T. 37.

Co-type in the Australian Museum, No. W. 367.

The largest specimen, fixed under slight pressure, measured 12 mm. long by 5 mm. broad; the smallest from the black toad-fish (Tetraodon hispidus ?) 8 mm. long by 3·75 mm. broad. Eight specimens were obtained from this host; but a large number of specimens, which differed from these only in being smaller in size, 5–6 mm. long by 2 mm. broad, were obtained from the intestine of the spotted toad-fish (Tetraodon hispidus). The worms are flat and almost rectangular in shape in the preserved specimens, the sides being fairly parallel and the ends almost truncated. The integument is thick and shows a number of corrugations on the surface. There are no spines.

The oral sucker is very large and strong, in many cases retaining within its grasp a piece of the mucous membrane of the host's intestine. It measures 1·666 mm. in diameter. The opening is terminal and comparatively small, with a condensation of the tissues round its edge so that the latter is specially tough. The ventral sucker is much smaller and weaker, and measures 0·714 mm. in diameter. The ratio of the oral to the ventral sucker is 7 : 3. The pharynx is a little smaller than the ventral sucker, and in most cases was compressed in the longitudinal direction. There is a distinct præ-pharynx, and a short œsophagus 0·12 mm. long (fig. 38) leading into the intestinal limbs, which run at first horizontally, where they bend round to become longitudinal; a cæcum is given off which extends forwards as far as the middle of the anterior sucker. The main posterior limbs, which are wide, extend straight back, parallel with the sides of the body, into the extreme posterior end. They have no branches, but in contracted specimens the walls are thrown into transverse folds.

The excretory pore opens at the posterior end, generally into a more or less deep depression. The vesicle is long and voluminous, extending as a wide straight tube as far forwards as the receptaculum seminis. Here it divides into two comparatively narrow vessels which runs forwards near the inner sides of the intestinal limbs and end blindly near the anterior end. From these vessels a number of capillary branches are given off.

The genital pore lies in the middle line, in front of the ventral sucker, and very close to its opening; in most cases almost on its lip. The testes lie in two parallel rows close to the inner border of the intestinal limbs. They are not only variable in number, but also unequal in number on the two sides. In eleven mounted specimens there were nine with five on the left side and six on the right; one specimen had three on the left and five on the right, while another had two on the left and three on the right. The two latter were not small nor immature, but amongst the largest of those present. The testes are not divided into double dorso-ventral rows like those of P. polyorchis Stoss., described by Linton in **26**, p. 460. Each testis is round and smooth, 0·476 mm. in diameter. The vesicula seminalis, lying close behind the ventral sucker, is large and pear-shaped. The ejaculatory duct, surrounded by a well-developed prostate, skirts round the left side of the ventral sucker, and, near its anterior border, opens into a tubular genital sinus. The latter, leading directly to the genital opening, in the form of a cylindrical tube, is 0·24 mm. long. There appears to be no cirrus sac; but the vesicula seminalis and the ejaculatory duct and prostate lie free in the body parenchyma.

The ovary is a smooth spherical body, 0·57 mm. in diameter, lying a little to the right of the middle line, near the anterior end of the testes. To its left is a large "shell-gland," and behind it a capacious receptaculum seminis. There is a Laurer's canal with comparatively thick walls. The uterus, running out laterally from the left side of the "shell-gland," is a comparatively small tube which reaches the level of the

anterior edge of the ventral sucker in three or four **S**-shaped bends, and opens into the genital sinus alongside the male opening. The eggs are never numerous. The yolk-glands, consisting of small rounded follicles 0·048 mm. in diameter, are very richly developed. In front they reach the level of the anterior border of the ventral sucker and are in this region confined to the lateral parts of the body. Passing backwards they gradually extend inwards to the middle line, so that in the posterior half of the animal they fill up the whole field, extending right across the body and back to the extreme posterior end. The eggs are thin-shelled, light in colour, 0·073–0·076 mm. long by 0·046 mm. wide.

This species differs from P. polyorchis Stossich in its larger size, in the absence of spines, in its much larger oral sucker, in the smaller number of testes, and in its spherical ovary.

Fam. Steringophoridæ.

Steringotrema pulchrum sp. n. (Figs. 12, 41 and 42).

Diagnosis.—Middle-sized worms, almost lanceolate in shape. Ventral sucker in front of the middle of the body. Ratio of the oral to the ventral sucker 1 : 1·8. Pharynx more than half as large as the oral sucker; œsophagus very short; intestinal limbs reaching to near the posterior end. Excretory vesicle **V**-shaped, the limbs reaching the level of the oral sucker in front. Testes near the middle of the body; ovary in front of right testis; Laurer's canal present, but no receptaculum seminis; loops of the uterus confined to the space between the intestinal limbs; yolk-glands lateral to the intestinal limbs, not reaching the ventral sucker in front; eggs 0·043–0·048 mm. long by 0·032–0·035 mm. broad, thick shelled.

In the gullet of the black toadfish,[1] and the spotted toadfish.[1]

[1] There seems to be some doubt whether these two fishes represent distinct species or colour varieties of the same species, Tetraodon hispidus.

Type-specimen in the Museum of the Australian Institute of Tropical Medicine, Townsville, No. T. 38.

Co-type in the Australian Museum, No. W. 368.

In size this species is 6 mm. long by 2·3 mm. broad, the greatest width being some distance behind the ventral sucker. The body is narrow in front of the ventral sucker, with fairly parallel sides ; the part posterior to it is oval in shape. The integument is thick and wrinkled, but no spines are present. As is usual in the group, the suckers are very large and strong, the longitudinal and transverse diameters of the oral sucker being 0·46 mm. and 0·58 mm. respectively, while those of the ventral sucker are $0·847 \times 0·978$ mm., the ratio of the oral to the ventral being $1 : 1·8$. The suckers are 1·3 mm. apart.

The pharynx is a conspicuous structure, but not so large as the oral sucker, measuring $0·342 \times 0·265$ mm. The œsophagus is very short, almost non-existent, while the intestinal limbs, which are somewhat voluminous, especially posteriorly, do not reach the posterior extremity by a distance of 1 mm.

The excretory vesicle may be described as V-shaped, and consists of two wide tubes extending from the level of the oral sucker to the extreme posterior end, where they are joined together by a short, transversely placed, triangular chamber which opens on the exterior at the posterior end. This part is surrounded by a mass of deeply staining cells. The two main limbs run at first along the inner side of the intestinal limbs. At the level of the testes they cross the intestine and proceed forwards, close to the sides of the body.

The genital pore is a little to the left of the middle line, just behind the pharynx. The two testes are almost symmetrically placed, one slightly in front of the other, and lie within the intestinal limbs. They are round or oval in shape and smooth-edged, 0·456 mm. in diameter. The vasa deferentia, after an elongated course, meet at the base of the vesicula seminalis, which lies wholly within the cirrus sac and is somewhat coiled. The prostate is well developed, and the penis large and thick. The cirrus sac is oval in shape, and its

walls are thick and muscular. The ovary is three-lobed, the lobes being somewhat indistinctly marked off by shallow grooves. It lies close in front of the right testis, and is smaller than that body, being 0·277 mm. in diameter. A small shell-gland lies on its left side in the middle line. There is a Laurer's canal, but no receptaculum seminis.

The coils of the uterus, in closely placed transverse loops on the left side, run back to the posterior end of the body— in the worm from which fig. 12 was drawn they do not extend so far back as usual—while the ascending loops keep mainly to the right of the middle line up to the level of the testes. From this point the uterus has a pretty straight course to the genital opening ; the vagina is not conspicuously developed. The coils of the uterus are entirely confined to the space between the intestinal limbs. The yolk-glands consist of from six to eight tree-like groups of small follicles on each side. The follicles are about 0·017 mm. in diameter, and there are 500 or 600 of them in each group. The number of groups on each side of the body is sometimes different. Each group opens by a separate duct into the longitudinal duct. The yolk-glands lie in the lateral field of the body outside the intestinal limbs, and extend forwards as far as the anterior border of the testes, whilst behind they extend a little beyond the middle point between the testes and the posterior end of the body.

The oval eggs are thick-shelled (fig. 12, a and b) with an operculum at the narrow end, while the broad end is often provided with a blunt spike. The eggs measure 0·043–0·048 mm. long by 0·032–0·035 mm. broad, and the shells are 0·0027 mm. thick.

A single immature specimen was found amongst the full-grown ones. It was 2·58 mm. long by 1·17 mm. broad. The suckers and pharynx bore the same relations in size as the mature worms. The testes measured 0·179 mm. in diameter; the ovary 0·098 mm. There were no eggs, nor could any trace of an uterus be made out.

In addition to the specimens from the gullet of the black

toadfish, a large number of specimens were obtained from the
gullet of the spotted toadfish, and they appear to me not to
differ from this species except in their smaller size. They
measured up to 4·25 mm. long by 1·78 mm. broad.

While I place this species in Odhner's genus Steringo-
trema, it appears to be more closely related to Distomum
vibex Linton (27, p. 291, figs. 48–51) than to any of the
three species enumerated by Odhner (51). Distomum
vibex Linton evidently belongs to the same genus. S.
pulchrum differs from Linton's species mainly in the dis-
position of the yolk-glands, in the shorter intestinal limbs, in
the more confined distribution of the uterine loops, and in the
size and shape of the eggs. It differs from S. cluthensis
Nicoll, S. pagelli van Ben. and S. divergens Rud.
in size, in the relative sizes of the suckers, in the very short
œsophagus, in having the post-acetabular considerably longer
than the pre-acetabular region, and in the very different
disposition of the yolk-glands, as well as differing from each
of the three species named in a number of other points.

Fam. Gorgoderidæ.

Sub-fam. Anaporrhutinæ Lss.

Petalodistomum[1] gen. nov.

Diagnosis.—Posterior part of the body very broad, almost
circular and plate-like. Muscular pharynx present;
short œsophagus. Genital pore at or behind the intestinal
fork. Cirrus sac very weak; testes deeply lobed and divided
into several distinct pieces, or broken up into a large number
of rounded follicles, lying wholly outside the intestinal
limbs. Large receptaculum seminis present but no Laurer's
canal. Yolk-glands lying wholly within the intestinal
limbs.

Type P. polycladum. Parasitic in the sting-ray, Dasy-
batis kuhlii.

[1] πεταλον, a plate.

The genus is closely related to Probolitrema Lss., differing from it principally in the fact that its yolk-glands are close together, lying within the space bounded by the intestinal limbs, while in the latter the yolk-glands are far apart and lie in the lateral part of the body quite outside the intestinal limbs (Looss, **33**, pp. 860 and 863, and Monticelli, **42**, tav v, fig. 52). It differs from Anaporrhutum Ofenheim in the testes being wholly outside the intestinal limbs.

Petalodistomum polycladum,[1] sp. n. (Fig. 13.)

Diagnosis.—Under middle size, petal-like, with the posterior part of the body almost circular. Ratio of oral to ventral sucker 1 : 1·6. Branched intestinal limbs. Genital pore at the level of the intestinal fork. Testes very large and more or less compact but divided into two or three pieces. Vesicula seminalis long, tubular and coiled. Ovary tri-lobed. Yolk-glands in two sets of small rounded follicles, close together, not extending outwards beyond the intestinal limbs.

Found in the body-cavity of the sting-ray, Dasybatis kuhlii.

Type-specimen in the Museum of the Australian Institute of Tropical Medicine, Townsville, No. T. 39.

Co-type in the Australian Museum, Sydney, No. W. 369.

Four specimens of this species, two of which were sectioned, were obtained from the body-cavity of the sting-ray. The posterior part of the body is almost circular, with a short blunt anterior part. It may be compared to the petal of a flower in which the lamina is circular and the claw short and blunt. The length of the animal varies from 3·3 to 3·76 mm., the breadth from 3 to 3·5 mm. The integument is smooth, without spines of any kind. The mouth-opening is terminal, the oral sucker bowl-shaped and deep, but the ventral sucker

[1] πολυς and κλαδος (branch), referring to the branches of the intestinal limbs.

is flat and shallow. The diameter of the oral sucker varies in the various specimens from 0·375 to 0·424 mm., while that of the ventral varies from 0·636 to 0·652 mm., the average ratio of the oral to the ventral being 1 : 1·6. The musculature of the body-walls is only poorly developed.

A strongly developed muscular pharynx, 0·25 mm. in diameter, leads directly out of the oral sucker, and is joined posteriorly by an œsophagus of rather less length, 0·195 mm. The intestinal limbs, which run some distance from the lateral edges of the body, roughly dividing it into thirds (fig. 13), do not quite reach the posterior end, and throughout their course give off about a dozen short branches from each side.

The excretory pore lies on the dorsal surface near the posterior end, and leads into a long tubular vesicle which runs forwards as far as the ovary, coursing dorsal to the uterus, and giving off a few small branches in its course.

The cerebral ganglia lie one on each side of the œsophagus, with the usual dorsal commissure and a pair of lateral cords that run backwards outside the intestinal limbs, giving off numerous short branches.

The genital pore lies on the ventral surface, in the middle line, just beneath the intestinal fork. The testes are very large, more or less compact bodies, lying wholly outside the intestinal limbs. From the posterior edge of the ventral sucker they extend backwards to within half their length of the posterior extremity. Each testis is divided up into two or three pieces, each of which is deeply lobed. The two vasa deferentia join near the middle of the ventral sucker and immediately enter the vesicula seminalis, which is long, tubular and coiled. The cirrus sac and ejaculatory duct are very poorly developed.

The ovary consists of three rounded lobes, and in its greatest diameter measures 0·326 mm. A small "shell-gland" lies on its left side, and just in front of it a receptaculum seminis, somewhat smaller than the ovary. The **yolk-glands** consist of fifteen to twenty small rounded

follicles (0·064 mm. in diameter) grouped in two fairly compact masses. They do not extend outwards beyond the inner edge of the intestinal limbs, but lie fairly close together, one on either side of the ovary. This does not quite agree with Looss' definition of the sub-family, "Dotterstöcke aus einander gerückt" (**33**, p. 863), and perhaps that definition is a little too narrow. The short, directly transverse yolk-ducts meet in a small yolk-reservoir lying ventral to the "shell-gland" and opening into the ootype by a comparatively long duct.

The coils of the uterus, never far from the middle longitudinal line, are confined laterally to the space within the intestinal limbs, but they extend a little further back, running out between the two ends of the intestine.

The eggs are rather long and narrow, 0·052–0·063 mm. in length by 0·023 mm. broad.

Petalodistomum cymatodes[1] sp. n. (Fig. 14.)

Diagnosis.—Above middle size, petal-like in shape. Oral and ventral suckers equal in size. Intestinal limbs unbranched but undulating. Genital pore behind the intestinal limbs. Testes consisting of a large number of widely diffused small follicles. Vesicula seminalis comparatively short. Ovary mulberry shaped. Yolk-glands, in the form of branching tubes, not extending outwards beyond the intestinal limbs.

Parasitic in the body-cavity of the leopard ray, Dasybatis kuhlii.

Type-specimen in the Museum of the Australian Institute of Tropical Medicine, Townsville, No. T. 40.

P. cymatodes, 10·5 mm. long by 8 mm. broad, is a good deal larger than its congener, but resembles it in shape. The oral and ventral suckers are about the same size, 1·14 mm. in diameter. The pharynx, which is obviously contracted in its longitudinal axis, measures 0·293 mm. by 0·538 mm. wide.

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[1] Κυματώδης, abounding in waves, referring to the intestinal limbs.

The intestinal limbs are unbranched, but are thrown into a number of snake-like undulations.

The genital pore is in the middle line, midway between the intestinal fork and the anterior edge of the ventral sucker. The vesicula seminalis and cirrus sac are short, and smaller than in P. polycladum.

The testes consist of about fifty small rounded follicles on each side, 0·107–0·129 mm. in diameter, and lying more or less dispersed from one another, in the region between the intestinal limbs and the lateral edges of the body. The ducts from the separate follicles join up in groups of five or six and enter a main longitudinal vas deferens on each side; these two vessels join one another at the base of the vesicula seminalis.

The ovary (0·375 by 0·244 mm.) is comparatively small, and is mulberry shaped. The receptaculum seminis is much larger, 0·73 × 0·57 mm.

The yolk-glands, on each side, consist of a much-branched tube rather than of follicles, and, while lying further apart than in P. polycladum, do not stretch outwards beyond the inner limit of the intestinal limbs. The extension of the uterus also coincides with that of P. polycladum. The eggs are larger, and especially broader than in the last-named species, being 0·06—0·064 in length by 0·03 mm. broad, and many of them are seen to be provided with a short spike at one end.

Only a single specimen of this worm was obtained from its host, the leopard ray, Dasybatis kuhlii, where it occurred in the body-cavity.

From the Australian Institute of Tropical Medicine and the Biological Department of the University of Sydney.

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EXPLANATION OF PLATES 22—27,

Illustrating Mr. S. J. Johnston's paper "On some Queensland Trematodes, with Anatomical Observations and Descriptions of New Species and Genera."

[The drawings, which were done by Mr. F. W. Atkins, of the Technical College, Sydney, were all made with the help of the camera lucida. The micro-photographs are from "untouched-up" negatives of sections and whole mounts.]

REFERENCE LETTERS.

c. g. Cerebral ganglion. *c. s.* Cirrus sac. *Ej. d.* Ejaculatory duct. *E.* Excretory vessel. *Ex. p.* Excretory pore. *Ex. v.* Excretory vesicle. *F. c.* Flame-cell. *G. p.* Genital pore. *G. s.* Genital sinus. *int.* Intestinal limbs. *L. c.* Laurer's canal. *L. t.* Lateral nerve-trunk. *M. o.* Mouth-opening. *N.* Nervous system. *Œs.* Œsophagus. *O. s.* Oral sucker. *O.* Ovary. *O. d.* Oviduct. *P.* Penis. *Ph.* Pharynx. *Pr.* Prostate. *R. s.* Receptaculum seminis. *S. g.* "Shell-gland." *Te.* Testis. *Ut.* Uterus. *Vag.* Vagina. *V. d.* Vas deferens. *V. s.* Vesicula seminalis. *V. Sk.* Ventral sucker. *Y. d.* Yolk-duct. *Y. g.* Yolk-gland. *Y. r.* Yolk-reservoir.

Rhabdiopœus taylori, figs. 1–4.

Fig. 1.—Drawn from a cleared and transparent object mounted with the dorsal side uppermost. × 8.

Fig. 2.—Posterior end, showing the multiple proboscis retracted into its complex sheath. × 21.

Fig. 2A.—Egg from the proximal part of the uterus. × 550.

Fig. 2B.—Egg from the distal part of the uterus. × 550.

Fig. 3.—Transverse section through one of the proboscides about its middle. × 110. *C. c.* Central cavity. *c. m.* Circular muscle-fibres. *G. c.* Gland-cell. *L. m.* Longitudinal muscle-fibres. *R.* Bundles of rhabdites.

Fig. 4.—Transverse section through proboscis near its tip. × 230. *R.* = single rhabdites.

Typhlocœlum reticulare, figs. 5-7.

Fig. 5.—Whole mount. × 30.

Fig. 6.—Excretory system, × 21. Compiled from a series of horizontal longitudinal sections, by the camera lucida. *F. c.* Flame-cells. *cap.* Capillary vessel. *net.* Network of vessels.

Fig. 7.—Nervous system, × 21. Compiled in the same manner and from the same series as used in fig. 6. *p. c.* Posterior commissure.

Fig. 8.—Allopyge antigones. × 7.

Fig. 9.—Diaschistorchis pandus. × 10. Unmounted specimen viewed by direct light.

Fig. 10.—D. pandus. × 20. *G. c.* Gland-cells surrounding the termination of the vagina.

Fig. 11.—Pleorchis oligorchis. × 14. Viewed as a transparent object from the dorsal aspect.

Fig. 12.—Steringotrema pulchrum. × 24.

Fig. 12A. Egg with operculum.

Fig. 12B. Egg from which the operculum has been removed. × 550.

Fig. 13.—Petalodistomum polycladum. × 21.

Fig. 14.—Petalodistomum cymatodes. × 11. *Ex. v.* Excretory vessel.

MICRO-PHOTOGRAPHS.

Rhabdiopœus taylori, figs. 15-26.

Fig. 15.—Part of a longitudinal sagittal section showing spines, thick ventral cuticle and the arrangement of the fibres in the muscular system. × 50. *C. m.* Circular muscle. *D. v. m.* Dorso-ventral muscle. *L. m.* Longitudinal muscle. *N. c.* Nerve-cord. *O. m.* Oblique muscle. *Sp.* Spine.

Fig. 16.—Part of a transverse section showing the arrangement of the muscle-fibres in the muscular system of the body and in the cirrus sac and vagina. The section also shows the very thick cuticle and the spines in transverse section. × 89.

Fig. 17.—Transverse section showing the bifid nature of the ventral spines. × 54.

Fig. 18.—Horizontal longitudinal section showing the relations of the nervous system, the alimentary canal and the excretory vessels. × 20.

Fig. 19.—Transverse section showing the brain. × 51.

Fig. 20.—Transverse section through the proboscis chamber and

tunnels, cutting one proboscis longitudinally and showing the tunnels or sheaths filled with mucus and rhabdites. × 53. *P. c.* Cavity in the proboscis. *M. r.* Mucus with rhabdites. *Ex. v.* Branches from the excretory vesicle.

Fig. 21.—Transverse section through the proboscis chamber, excretory vesicle and excretory pore, showing the relations of the excretory vesicle to the proboscis chamber. × 45. *Pb. ch.* Proboscis chamber.

Fig. 22.—Transverse section a little further forward than the two foregoing, showing the excretory vessel leading from the vesicle into the central cavity of one of the arms of the proboscis. × 45. *Pb.* Proboscis, cut obliquely.

Fig. 23.—Transverse section passing through the three anterior arms of the proboscis, showing them lying in their sheaths or tunnels. × 20. *Pb. s.* Proboscis sheath or tunnel.

Fig. 24.—Transverse section cutting the cirrus sac in the region of the vesicula seminalis. × 73.

Fig. 25. Transverse section through the cirrus sac in the region of the prostate. × 85.

Fig. 26.—Horizontal longitudinal section showing the anterior arch of the excretory vessels. × 70.

Typhlocœlum reticulare, figs. 27–30 and 33.

Fig. 27.—Horizontal longitudinal section showing nerve-cords and ventral network of nerves. × 70.

Fig. 28.—Photograph of whole mount, focussed about the middle of its thickness, showing the characteristic dorso-ventral winding of the uterus. × 20.

Fig. 29.—Horizontal section through the anterior end, showing the relations of the cerebral ganglia and pharynx. × 53.

Fig. 30.—Transverse section about the middle of the body, showing the characteristic coils of the uterus. × 53.

Allopyge antigones, figs. 31 and 32.

Fig. 31.—Transverse section (somewhat oblique) through the pharynx and base of the "oral sucker," showing the muscle-fibres in the walls of the latter. × 56.

Fig. 32.—Transverse section in the region of the ovary, showing the receptaculum seminis and "shell-gland." × 45.

Fig. 33. — Typhlocœlum reticulare, horizontal longitudinal section, showing the network of excretory vessels. × 60.

Diaschistorchis pandus, figs. 34-37.

Fig. 34.—Horizontal longitudinal section, showing alimentary canal, cirrus sac and the related parts, uterus and yolk-glands.　× 48.

Fig. 35.—Part of a transverse section passing through the genital aperture, showing the genital sinus, vagina and penis.　× 82.

Fig. 36.—Part of a transverse section passing through the vagina and the cirrus sac in the region of the ejaculatory duct, showing the very thick muscular wall of the sac.　× 51.

Fig. 37.—Horizontal longitudinal section, showing the separate testes and general anatomy.　× 15.

Pleorchis oligorchis, figs. 38-40.

Fig. 38.—Horizontal longitudinal section, showing alimentary canal (partly filled up with host's blood), œsophagus, excretory vesicle and main relations of the reproductive organs.　× 24.

Fig. 39.—Part of transverse section passing through the ventral sucker near its posterior edge, and showing the genital sinus, vagina and male duct, with prostate lying free in the body parenchyma.　× 51.

Fig. 40.—Transverse section in the region of the ovary, showing ovary, "shell - gland," yolk-duct, oviduct, receptaculum seminis and Laurer's canal.　× 51.

Steringotrema pulchrum, figs. 41 and 42.

Fig. 41.—Longitudinal sagittal section a little to one side of the median plane, showing general anatomy.　× 26.

Fig. 42.—Transverse section in region of the ovary, showing Laurer's canal, oviduct, etc., and the relations of the intestinal and excretory tubes.　× 53.

On the Maxillary Glands and some other Features in the Internal Anatomy of Squilla.

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With Plate 28 and 9 Text-figures.

Contents.

I. The Maxillary Glands.

Preliminary Comments.

THE following description of the maxillary gland of Squilla was intended to form but a small part of a monograph dealing

with the anatomy in general of this aberrant Crustacean, but in consequence of my appointment to the Chair of Zoology at the Muir Central College, Allahabad, this project of a monograph has had to be abandoned. Since the description, so far as it goes, is fairly complete and intelligible, I have thought it worth while to publish it, together with a few notes on other parts of the anatomy of Squilla.

As is well known, the general text-book statement that antennal glands are characteristic of adult Malacostraca in the same way that maxillary glands are of " Entomostraca " now admits of many exceptions, since maxillary glands have been found in a number of undoubted Malacostracan genera. Maxillary glands have been described in adult forms of Leptostraca (Nebalia, Claus [7]), of Syncarida (Anaspides [3]), of Tanaidacea (Apseudes, Claus [8]), of Isopoda (Bopyrus, Gyge, Porcellio, Ligia, Asellus, Rogenhofer [21]), and of Stomatopoda (Squilla, Kowalevsky [14]). Since the researches of Grobben (10), Marchal (17), Kingsley (13), Waite (23), and others have demonstrated the high degree of complexity attained by the antennal glands in the higher and larger Malacostraca, it is perhaps surprising that the condition of the glands in the large-sized and, in many respects, highly developed and aberrant Squillidæ should not hitherto have attracted the attention of zoologists, especially in view of the statement of Kowalevsky (14) that the glands are maxillary and not antennal. However, apart from the rough figure by Claus (4, Taf. iv, fig. 8) of the maxillary glands in a late Stomatopod larva, the bald statement of Kowalevsky that they exist in the adult Squilla, and the preliminary description of these glands which I read (25) before Section D at the 1911 meeting of the British Association for the Advancement of Science (Portsmouth), nothing has been published on the subject.

As material for this inquiry, I employed adult specimens of Squilla desmarestii (Risso) and Erichthus larvæ of Lysiosquilla eusebia (2 mm. in length, and comparable with the figures 191 A and B shown in Calman [3]), obtained

from Naples and fixed and preserved by the methods
enumerated in the Appendix; also well-fixed Erichthus larvæ
of an unknown species of Squilla, 12 mm. and 14 mm. in
length respectively, preserved in the store-room at University
College, London. Several complete series of transverse and
longitudinal vertical sections were made of both adult and
larval specimens, and the following description is based upon
the careful study of these sections, assisted by dissections
both of adult Squilla desmarestii and Squilla mantis
and whole mounts of the smaller larvæ.

Before proceeding further I wish to offer my sincere thanks
to Dr. W. T. Calman for kindly seeing the MS. through the
press, and to Miss E. M. Brown for the careful drawings
which illustrate the paper.

The Maxillary Glands of the Adult Squilla Desmarestii.

Macroscopic Appearance.

The paired maxillary glands lie at the hinder end of the
" neck " of the animal, nearly on a level with the proximal
joint and epipodite of the large subchelate second thoracic
limb. Previous to dissection they may be seen through the
transparent cuticle covering the sides of the neck as large
yellowish masses, provided that the anterior thoracic limbs
be pulled outwards from their forwardly directed position
under the sides of the carapace. On removal of the dorsal
carapace the paired glands are found to lie just under the
hypodermis. Superficially, the glands closely resemble a pair
of adductor muscles (the vertically descending gland-ducts
simulating tendons) connected with the mandibles (Pl. 28,
fig. 1), and it seems probable that they have been identified
as such by previous observers, since otherwise it is difficult
to understand how such conspicuous structures could escape
attention. Viewed from the dorsal aspect, each of the two
glands is seen to be a pear-shaped, compact yellowish mass,
which tapers ventrally into a thin stalk, the stalks of the two
glands converging towards the median line and disappearing

one on each side of the large sub-œsophageal compound
ganglion (*U. Th. G.*, Pl. 28, fig. 1). On closer inspection (Pl.
28, fig. 2) the thick upper portion of the pear-shaped gland is
seen to be deeply grooved on its outer side to allow space for
a large body-muscle which happens to traverse the body-
cavity in the proximity of the gland. Although the gland is,
in parts, closely wrapped round the muscle, yet there is, of
course, no organic connection between the two structures
In fig. 2 (Pl. 28) the duct of the gland is seen to enter the
base of the maxilla (the second maxilla), and, after dilating
slightly to form a bladder, to open on the extremity of the
small papilla already described by Calman (**3**), who had pre-
viously suggested that the minute orifice present might prove
to be that of the maxillary gland. In fig. 2 (Pl. 28) are also
shown the numerous minute flask-shaped multicellular glands
which are to be found scattered in many other regions under
the hypodermis. These glands are connected with the setæ
borne on the cuticle, and have been previously figured and
described (under the general name of "drüsenzellen," and
often incorrectly stated to be unicellular glands) by Claus (**5**),
Jurich (**12**) and others. A drawing of a section through one
of these glands (showing the gland lumen) is shown in Text-
fig. 8. Fig. 2 (Pl. 28) represents the isolated maxilla and the
attached gland as seen in spirit under the dissecting micro-
scope ; when in balsam the numerous seta glands are usually
almost invisible, since staining reagents rarely penetrate
sufficiently in order to render them conspicuous. The minute
papilla with its gland opening is situated on the convex side
of the maxilla, i. e. away from the mouth. The gland itself
is, as we shall see, closely invested with squamous epithelium,
and in size is about 4 mm. long, excluding the duct and the
bladder, and about 2·5 mm. in maximum width.

General Histology.

The general position in the body and histological construc-
tion of the gland may be gathered from a perusal of fig. 3

(Pl. 28), which represents the gland and other organs in a transverse section of the left side of this region of the body. Most of the hæmocœle in this region is seen to be occupied

TEXT-FIG. 1.
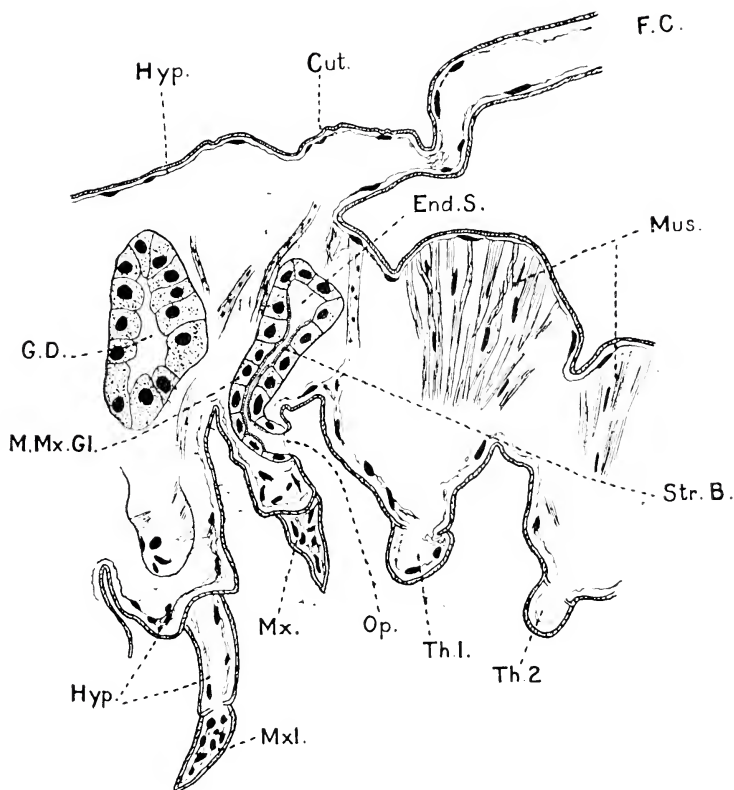
Maxillary gland in a sagittal section of the larva of Lysiosquilla eusebia, 2 mm. long. *End. S.* Future end-sac. *M. Mx. Gl.* Future kidney. *Op.* Opening of gland duct. *Str. B.* Striated border of gland-cells of kidney portion (absent in cells of end-sac). (× 384.) For other reference letters see p. 429.

by large oblique longitudinal and transverse muscle bands and the maxillary glands. The parts of the gland shown in this figure, however, do not all occur in any one of my thin sections, but extend through several—the figure may be

our material the entire gland consists of a simple deep pocket, somewhat dilated at its blind extremity, which has apparently been formed by an involution of the hypodermis, i.e. the ectoderm (Text-fig. 1). At a later stage the gland consists of two wide sacs which only communicate with each other by a narrow tube—the distal or " end-sac " and the

TEXT-FIG. 3.
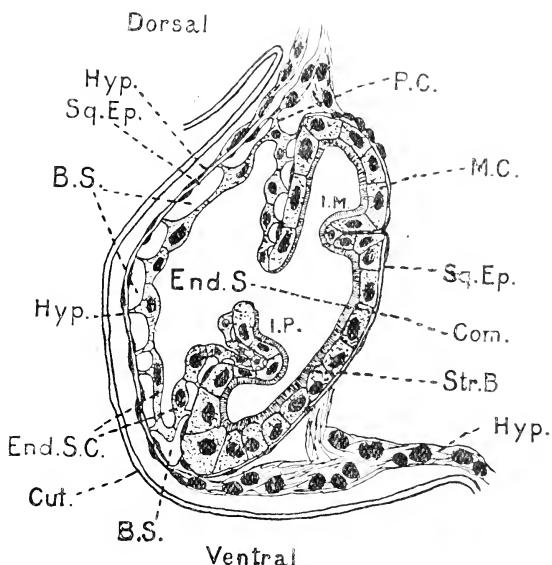
Transverse section across the end-sac and terminal part of the kidney of the gland (in a transverse section of a 14 mm. larva) showing the connection (*Com.*) between the two. *Cut.* Cuticle. *Hyp.* Hypodermis. *Sq. Ep.* Squamous epithelium underlying hypodermis. *End. S. C.* End-sac cells. *B. S.* Blood-sinus (contained blood not shown). *P. C.* Protoplasmic processes from end-sac cells extending across blood-space to squamous epithelium. *I. M.* Invagination of kidney wall in process of formation. *I. P.* Similar invagination of septum separating end-sac from kidney. (× 384.) For other reference letters see p. 429.

proximal sac or gland proper or k i d n e y, as I shall term it in future, which opens to the exterior v i â the duct (Text-figs. 2 and 3). During subsequent development each of these divisions, but especially the kidney, enlarges greatly, and

TEXT-FIG. 4.
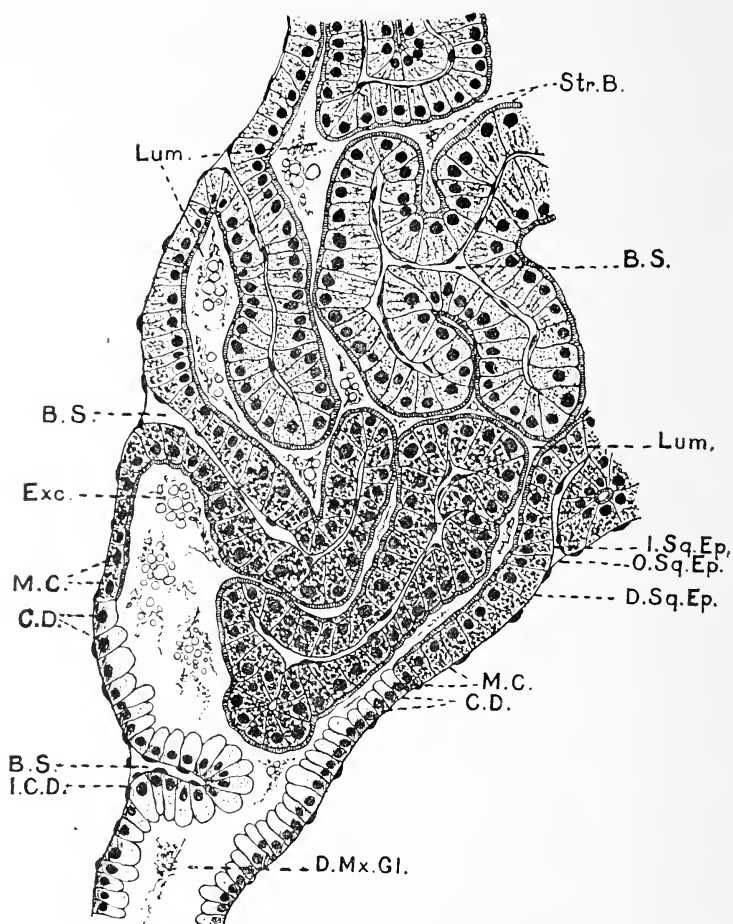
Structure and histology of kidney and proximal duct portion of the gland in the adult Squilla. *Lum.* Lumen of gland containing excretory matter (*Exc.*). *B. S.* Blood-sinus (morphologically external to gland). *O. Sq. Ep.* Outer layer of squamous epithelium forming external wall of blood-sinus. *I. Sq. Ep.* Inner layer of squamous epithelium (forming inner wall of blood-sinus). *D. Sq. Ep.* The double layer of squamous epithelium. *I. C. D.* Invagination of duct-cells. Note absolute distinction between duct (*C. D.*) and kidney cells (*M. C.*) with striated border (*Str. B.*)—intermediate types do not exist. (× 296.)

simultaneously their lumina become invaded by numerous invaginations of their walls (two incipient invaginations are shown in Text-fig. 3). These invaginations are formed at all points of the walls of the enlarged kidney and end-sac and become so long, branched and closely compacted internally in the former that the originally spacious lumen is reduced in all parts to thin cracks lying between adjacent cell-layers (Text-fig. 4). The entire exterior of the gland is invested with two layers of squamous epithelium, between which lies a division of the hæmocœle. The inner layer (*I. Sq. Ep.,* Text-fig. 4) only is closely applied to the surface of the entire gland, and becomes involved in all the invaginations just referred to. Careful inspection of well-stained sections shows (Text-fig. 4), as might be inferred from the development of the gland just outlined, that the mass of the gland exhibits two kinds of spaces : (i) Spaces hæmocœlic in nature, and therefore morphologically outside the gland, lined by the squamous epithelium, which originally covered the gland surface and which subsequently followed the invaginations of the gland wall ; and (ii) spaces devoid of a squamous epithelium, being solely bordered by the gland-cells, and representing all that is left of the originally wide lumen of the gland. Thus, it will be seen that the narrow blood-space surrounding the gland penetrates by means of the invaginations described into the innermost parts of the mass of the organ, and in every part is only separated from the lumen of the gland by a thin squamous epithelium and the single layer of gland-cells which purify the blood. The squamous epithelium forming the outer wall (*O. Sq. Ep.*) of the hæmocœle, and lying wholly on the periphery of the gland, lies for the most part in close contact with the inner squamous layer (*I. Sq. Ep.*), where this is situated at the periphery, and in my sections can only be distinguished from it at those points where the inner layer becomes invaginated to line the involutions of the gland wall, and so necessarily becomes separated (Text-fig. 4).

In longitudinal sections of the gland and duct (Pl. 28, fig. 3), the three regions characteristic of Crustacean antennal

and maxillary glands can be distinguished : (a) The region of the end-sac, (b) the region of the kidney, and (c) the region of the duct.

Histology of the End-sac.

The region of the end-sac is distinguishable from the region of the gland proper by its relatively small size as compared with the kidney (though early in development they are equal

TEXT-FIG. 5.
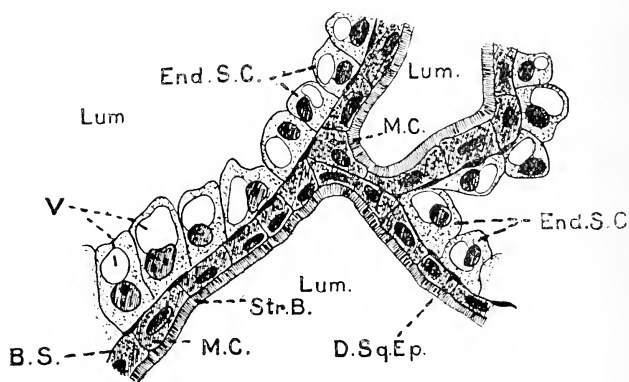
Cells of end-sac (*End. S. C.*) showing vacuoles (*V.*) and slightly granulated cells of kidney (*M. C.*) next end-sac (from longitudinal vertical section of adult Squilla). *B. S.* Very narrow blood-sinus. (× 810.) For other reference letters see p. 429.

in size), by its spacious lumen (the invaginations of the end-sac wall being few in number and simple in character) and by the character of the cells. The position of the narrow communication between the lumen of the end-sac and the kidney is very difficult to detect, since the opening is apparently not made evident by the presence of a couple or more of large " trichterzellen," such as those described by Allen (1) in the maxillary gland of the larva of Palæmonetes and by Vejdovský (22) in the antennal gland of Gammarus, and it is thus almost impossible to distinguish this narrow channel from the numerous other narrow channels penetrating the

entire mass of the gland. If Text-fig. 3 be referred to, it will
be seen that in the early stage of development figured, the
septum (i. e. the apposed walls) separating the end-sac from
the kidney is giving rise to an invagination in the same way
as the other parts of the gland-wall, and hence the boundary
between these two parts of the gland becomes very sinuous
in the fully formed organ (Pl. 28, fig. 3), though easily dis-
tinguishable on account of the characteristic features of the
end-sac cells. The end-sac as a whole is situated on the
upper and slightly posterior and external lateral aspect of the
fully formed gland, i. e. it retains the position in which it
arose. The constituent cells of the end-sac of the fully
formed gland (Text-fig. 5) are somewhat irregular in outline
and consist of faintly granular cytoplasm, which is difficult to
stain, and large nuclei. In close apposition to the nucleus
of each cell is a large and very characteristic vacuole (V),
usually much larger than the nucleus. These cells of the end-
sac differ markedly from those of the gland proper, and the
two kinds of cells are quite distinct—there are no cells
transitional in character. The cells of the fully formed end-
sac also differ greatly from those of the larval end-sac, as will
be seen below. The blood-sinus, which extends into the
septum separating the end-sac from the gland proper, is so
attenuated in the gland of the adult as to be only recognis-
able in position by the nuclei of the two opposed layers of
squamous epithelium composing its walls.

Histology of the Kidney or Gland Proper.

As already stated, in the region of the kidney, the wide
lumen of the original sac is reduced to slit-like spaces
situated between the crowded invaginations of the wall
already described (Text-fig. 4). These thin spaces represent-
ing the gland lumen can always be distinguished from the
equally thin extensions of the external hæmocœle by the
facts that (A) the excretory matter contained in the lumen is
largely in the form of spherical bubble-like masses (the blood,

on the other hand, appearing as a fine sediment or homo-
geneous film), and (B) that they are lined, not by squamous
epithelium, but by the excretory cells, the border of which,
situated next the lumen, is characteristically striped. The

TEXT-FIG. 6.
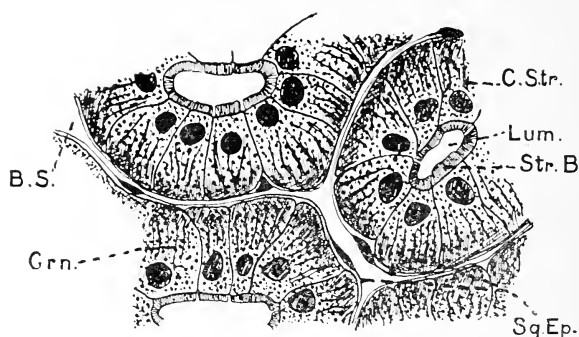
Cells of middle portion of kidney showing least amount of
granulation, also striated border (*Str. B.*) and striations in
cytoplasm (*C. Str.*). Blood-sinus (*B. S.*) relatively spacious.
(× 810.)

TEXT-FIG. 7.
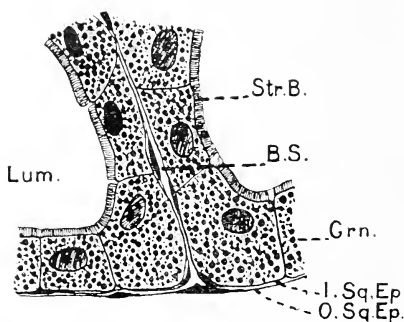
Cells of kidney nearest duct showing greater granulation (*Grn.*).
(× 810.)   For other reference letters see p. 429.

cells composing the kidney vary slightly in character accord-
ing to their position, those situated towards the end-sac, i. e.
in the dorsal region, being far less granular and somewhat
more columnar in form than the cells situated nearer the
gland-duct. The more dorsally situated cells (Text-fig. 6)

are large and columnar in form. The cytoplasm contains numerous small granules, is somewhat denser in the region next the hæmocœle, and shows a faint striation parallel with the cell length. The portion of the cytoplasm bordering the lumen is, as above stated, characteristically striped. The nuclei are always situated near the striated border, and are large, with conspicuous chromatin granules. These less granular columnar dorsal cells pass gradually into the more granular and often flatter cells found towards the duct end of the gland (Text-fig. 7). The ventral cells chiefly differ from the dorsal in that the granules contained in the cytoplasm are more numerous and are much larger and that there are no striations in the general cytoplasm. It should also be mentioned that the cells composing the part of the kidney actually in contact with the end-sac are flat and small (Text-fig. 5), and that these gradually merge into the columnar kidney-cells already described.

Histology of the Duct.

The duct region of the gland is distinguishable as a whole from the region of the kidney by the spacious lumen (a few wall invaginations only being present in the upper part of the duct) and by the character of the cells composing its wall. The character of the cells of the duct, indeed, enables this region to be distinguished with the greatest precision from the adjoining region of the gland proper, since, as can be seen in Text-fig. 4, there exist no cells intermediate in character between those composing the duct, on the one hand, and those composing the kidney on the other. We saw that the region of the gland proper could be sharply distinguished by the same means from the region of the end-sac, the cells of the latter being quite distinct in character.

The duct region of the gland can (Pl. 28, fig. 3) be subdivided into three sub-regions : (1) The upper duct, (2) the bladder, and (3) the duct aperture to the exterior. In the subregion of the upper duct the lumen is wide, contains a

large quantity of excretory products, and is only slightly
invaded by a relatively few simple wall-involutions (Pl. 28,
fig. 3). The character of the cells forming the duct wall is
shown in Text-fig. 4. The cytoplasm is faintly stained on the
side situated next the lumen, but denser in appearance next
the hæmocœle and is throughout faintly granular. The
nuclei are large and spherical. In shape the cells are very
irregular, and are quite devoid of the conspicuous striated
border so characteristic of the cells of the gland proper. The
nuclei, like those of the gland-cells, are situated at one end
of the cell, but unlike those of the kidney, are situated next
the hæmocœle and not next the lumen. Text-fig. 4 shows well
the respective characters of the duct- and gland-cells and the
abrupt transition from the one type to the other. Judging
from the character of the cells of the duct, it seems probable
that they take little or no share in the process of excretion.
In the sub-region of the bladder the lumen is more spacious
than in any other part of the gland and invaginations of the
wall are quite absent. The cells of the walls are short,
columnar or cubical in form (Text-fig. 8), and the cytoplasm
is uniformly pale and faintly granular ; there is no polar
differentiation as in the cells of the upper duct. The nuclei
are situated at the ends of the cells remote from the lumen.
It is perhaps important to notice that the walls of the bladder
near the external opening (Text-fig. 8) are devoid of the
double layer of investing squamous epithelium found on the
exterior of all other parts of the gland—a fact possibly of signifi-
cance in connection with the question as to the germ-layer
origin of the gland to be discussed later.

    The bladder rapidly becomes narrow at its lower end to
open to the exterior (Text-fig. 8). A notable feature of this
external opening is the modification of the cells lining the
inner and posterior side of the duct. As will be seen from
fig. 3 (Pl. 28) and Text-fig. 8, the cells in the position stated
become enormously elongated in a direction approximately
parallel with the length of the duct to form a conspicuous
clump of columnar cells continuous at the upper end with the

cells of the bladder-wall and at the lower end with the cells of
the hypodermis (the hypodermis has shrunk away from the
cuticle all round the opening of the duct to the exterior). This
clump of cells thus does not appear to form an ordinary
sphincter muscle, since the muscle-fibres are not disposed

TEXT-FIG. 8.
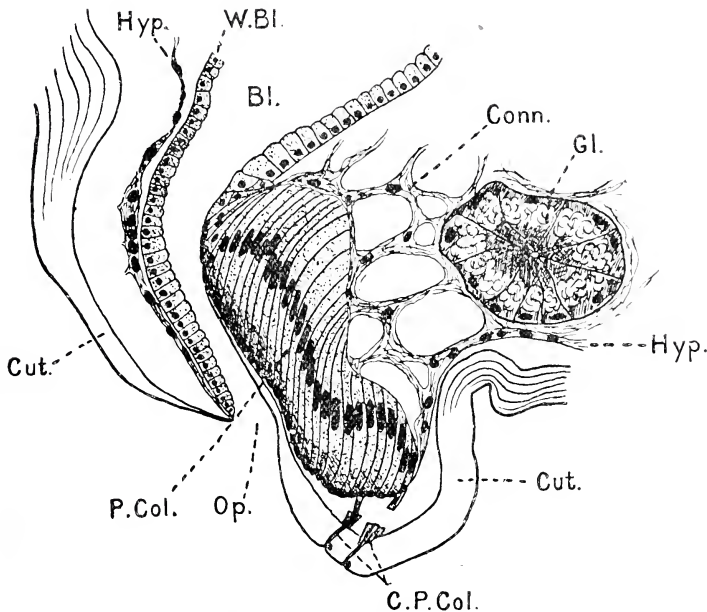
Opening of the gland. *Bl.* Lower portion of bladder. *Op.*
Opening of duct. *P. Col.* Patch of columnar epithelium at duct
opening, the lower cells of which are, in life, connected (*C. P. Col.*)
with the two curious plug-like bodies situated in the cuticle
(*Cut.*). *Gl.* Transverse section across one of the numerous
small multicellular seta glands (drüsenzellen). The hypodermis
has shrunk away from the cuticle. (× 384). For other
reference letters see p. 429.

circularly round the narrow duct and they are only developed
in the inner wall. If this clump of cells be examined carefully
it is seen that the outer ends of most of these cells (containing
denser cytoplasm) are in close contact with a thick strip of
cuticle which extends up the inner wall of the duct and
nowhere else, and that those cells situated at the extreme end

of this duct opening (which have presumably shrunk away from the thick cuticle) are also connected with the cuticle by protoplasmic processes (nearly a dozen in number, though only about six are shown in the drawing) developed from their outer ends, which converge towards and become inserted into two curious plug-like bodies firmly imbedded in the cuticle (*C. P. Col.*, Text-fig. 8). These two plug-like bodies are not found in any other part of the body, and doubtless possess some special retentive function in connection with the cell-clump here developed. At their inner ends the cells composing the clump are ill-defined, and appear to merge into the numerous connective-tissue strands (*Conn.*, Text-fig. 8) occupying the cavity of the maxilla in this region. It is possible that the cells of the clump represent smooth muscle-fibres (they are not striped transversely), which on contraction serve to block up the external aperture of the gland. It is certain that the clump is not glandular, developed for the purpose of secreting the extra thick cuticle in this region, since the ordinary hypodermis is not specially developed elsewhere to produce this result, and it is difficult to suppose that the clump possesses a sensory function, though the pigment (?) spots associated with the two " plugs " at first suggest this.

Some Stages in the Development of the Maxillary Gland.

The Gland of the 2 mm. Larva of Lysiosquilla eusebia. — In some Erichthus larvæ of Lysiosquilla eusebia, 2 mm. in total length, comparable with the developmental stage figured, after Claus, by Calman (**3**) in his fig. 191A (only the second thoracic limb in my specimens has not yet become enlarged), and, with the exception of the " metanauplius " stage of Lister (**16**), representing the youngest larval form of Squilla yet known, the maxillary gland is of the simple form represented in Text-fig. 1. The gland at this early stage of development solely consists of a simple, deep, pocket-like ingrowth opening at the base of the maxilla on

its posterior outer side and possessing a blind extremity which
is somewhat dilated. This blind extremity lies just under
the junction of the free carapace (*F. C.*) with the body and
just behind the diverticulum of the gut (*G. D.*) which will
subsequently form the "liver." Text-fig. 1 is plano-stereo-
scopic (combines in one plane parts which are only to be
found in separate sections and which would only be visible
collectively under the microscope if the section were of
sufficient thickness to contain them all), and represents the
gland as seen in sagittal sections of the larva. In a series of
transverse sections of the larva, the gland was only included
in eight successive sections, each 10 µ thick, and appeared as
a small tube cut transversely owing to the gland slanting
posteriorly in position. The gland is, in all probability,
functional at this early stage of development, since, with the
exception of those situated at the extreme end of the diverti-
culum and at the external opening, all the cells (in the
middle part of the gland labelled *M. Mx. Gl.*) possess the
striated border so characteristic of the excretory cells of the
gland proper in the fully formed organ and contain granules
in the cytoplasm. The fact (perhaps I should say probability,
since the sections do not afford quite conclusive evidence)
that the extreme cells of the terminal dilatation do not possess
a striated border certainly favours the conclusion that this
region becomes the end-sac of the fully formed gland. If
this last statement be true (and there is but little doubt
about it), then, in view of the obvious continuity of the wall
of the gland diverticulum with the hypodermis at the external
opening (see Text-figs. 1 and 8) and the absence of mesen-
chymatous cells in the vicinity of the gland (the gland is
apparently not invested by squamous epithelium at this
stage), it seems extremely probable that the whole of the
diverticulum (and therefore the whole of the fully formed
gland, end-sac and all) is the product of an involution of the
hypodermis, i.e. ectoderm. The probability of this conjec-
ture is discussed below.

The Gland in a 12 mm. Larva, Squilla sp.—The stage

of development of the maxillary gland in this larva is indicated in Text-fig. 2, A and B, which represents a reconstructed stereoscopic view of the gland based on a series of drawings, drawn to scale, taken from the serial sections. In this figure the gland is viewed from the dorsal aspect (cf. Pl. 28, fig. 1, showing the adult gland). In A the three parts of the gland, which are now easily distinguishable, are shown in their correct relative positions. The end-sac (*End S.*) is shown to lie on the postero-dorsal aspect of, and in close apposition with, the kidney (*M. Mx. Gl.*), and the latter is, of course, continuous with the vertically descending duct (*D. Mx. Gl.*), the terminal part of which is not yet dilated to form a bladder. In B the end-sac is supposed to have been pulled apart from the kidney in order to show the posterior position of the narrow canal (*Com.*) putting the two parts into communication.

The Gland in a 14 mm. Larva, Squilla sp. — Though the Erichthus larva of this size is not much larger than that of the preceding stage, and the maxillary glands of both stages are practically identical, yet the gland of the older larva differs in one small though important feature from that of the younger. In a transverse section of the gland at this stage of development (Text-fig. 3) passing through the narrow communication (*Com.*) between the end-sac and the kidney, the cavities and walls of these two regions are well shown. The cells composing the wall of the cavity representing the kidney (*Mc.*) are very similar to those found in the gland of the adult Squilla. They are regular in outline and stain deeply; the cytoplasm is granular, and there is a well-defined striated border present (*Str. B.*). The quite different cells composing the wall of the end-sac (*End. S. C.*) are, on the other hand, very dissimilar to those of the fully formed gland. As shown in the figure the faintly granular cytoplasm is drawn out at irregular intervals into tent-like projections (*P. C.*), which stretch across the intervening space (hæmocœle) to the layer of squamous epithelium surrounding the whole gland. These projections are apparently found in connection

with the end-sacs of most antennal and maxillary glands, since they are figured by Grobben (**10**), Claus (**6**), Vejdovský (**22**), and many other authors. Finally, it will be noticed that these end-sac cells contain none of the vacuoles so characteristic of the cells composing the fully formed end-sac.

As regards the squamous epithelial investment of the gland at this stage of development it will be remembered that in the adult Squilla the whole of the gland, excepting the lower part of the bladder, is enclosed in a double layer of squamous epithelium, the two layers enclosing a blood-space. On the other hand, in the early stage of development of the gland depicted in Text-fig. 1 no epithelial investment whatever can be observed. Text-fig. 3 shows what may perhaps be regarded as an intermediate condition. As will be seen, the gland at this stage lies in close contact with the side of the body, and is only separated from the hypodermis by a (apparently) single layer of squamous epithelium (*Sq. Ep.*), which is also to be found on the inner side of the gland, and penetrates between the closely apposed walls of the end-sac and the gland proper. This single layer of squamous epithelium also penetrates into the invaginations of the wall, the presence of which distinguishes the gland of the 14 mm. larva from that of the 12 mm. In this 14 mm. larva, therefore, we can observe the commencement of the formation of those involutions of the wall of the gland proper (two are shown in Text-fig. 3—*I.M.* and *I.P.*), which ultimately so invade the cavity of this region as to reduce it to the winding system of narrow cracks already described as characteristic of the fully formed organ. Since the single layer of investing squamous epithelium is drawn into each of these involutions of the wall, it must therefore represent the inner layer of the two layers characteristic of the adult condition. The outer layer of squamous cells of the adult gland is possibly produced later by the splitting (delamination) of the inner (I admit that the only evidence I have for this suggestion is the occasional appearance, in sections, of a double condition to be found

over small areas of the single squamous layer.) The duct
to the exterior is composed of cells similar to those of the
gland proper, save that they lack the striated border. There
is as yet no dilatation to form a bladder.

Remarks on the Maxillary Gland of Squilla compared with the Excretory Leg Glands of other Crustacea and Arthropoda.

If we compare the maxillary glands of Squilla with those
of the Isopoda (described by Rogenhofer [21]) it is at once
evident that the former greatly exceed the latter in com-
plexity of structure, and in fact the maxillary glands of the
adult Bopyrus, Porcellio, and even Ligia are almost exactly
comparable, both as regards general form and histology, with
the early stage of development of the maxillary gland found
in the 12 mm. larva of Squilla. In Asellus aquaticus, on
the other hand, the kidney region of the gland has, according
to Rogenhofer, become much lengthened and convoluted and
therefore more similar to the maxillary gland of Branchipus
(Claus [6]) or the antennal glands of Gammarus (Vejdovský [22]
and Mysis (Grobben [10]). In connection with the antennal
and maxillary glands of Malacostraca generally it appears
that the kidney part of the gland is able to undergo compli-
cation in two different ways: (1) It may either elongate and
become greatly convoluted (Asellus, Gammarus, Mysis), or
(2) it may swell and its wall become invaginated by inward
extensions of the external hæmocœle. As examples of the
latter method may be mentioned the maxillary gland of
Squilla and the antennal glands of Cancer (Pearson [18]),
Homarus (Waite [23]), Palæmonetes (Allen [1]), etc. I should
like here to record my suspicion that many descriptions (e.g.
Waite's description of the antennal gland of Homarus ([23])
and Weldon's description of the kidney region of certain other
Decapods [24]) of the structure of the kidney region of the
antennal gland in different genera of Malacostraca commit the
error of describing as an elongated convoluted duct what should

be described as the extensive invagination of a swollen sac. In both cases sections would reveal tubular structures cut across, and it is only by careful reconstruction and examination of the investing epithelia that the observer can be certain as to which type of structure his sections represent.

Concerning the comparative histology of the maxillary gland of Squilla, I must remark that in general it resembles that of the antennal and maxillary gland of other Crustacea, as reference to the works of Grobben, Rogenhofen, Allen, Vejdovský and others will show.

As regards the germ-layer origin of the antennal and maxillary glands in Crustacea, it is interesting to note the differences of opinion among authors on the subject. For instance, my figure of the youngest stage of development of the maxillary gland of Squilla closely resembles those of Ishikawa ([11], his fig. 92) and Grobben (10), representing the early development of the antennal glands of Atyephira and Cetochilus respectively, but whereas Ishikawa states that the young gland is ectodermal in origin, Grobben holds that it is mesodermal, arising as an invagination of the somatopleure. Reichenbach (19) held that the antennal glands of Astacus are purely ectodermal structures, and a number of later authors agree that at least the external part of the gland in the Crustacea is of ectodermal origin, e. g. Ishikawa (11) believes that the entire gland of Atyephira is ectodermal, whilst Lebedinsky (15), Boutchinsky (2) and Waite (23) state that the duct and kidney are ectodermal, the end-sac being mesodermal. On the other hand, Grobben (10), Kingsley (13) and Robinson (20) consider the whole gland to be a mesodermal product, but Grobben's statement concerning the somatopleuric invagination is certainly difficult to believe, since a cœlomoduct is formed as an outpushing, not an ingrowth, and Robinson's figures of the first rudiments of the antennal gland in Nebalia show that the initial clump of cells may just as well be considered ectodermal as mesodermal. It must be concluded, I think, that the evidence for the ectodermal origin of the duct and kidney of the gland is pre-

ponderant. Concerning the origin of the end-sac, the evidence is not so one-sided. The statements of Waite, confirming those of Lebedinsky and Boutchinsky, are quite definite as to the origin of the end-sac in a distinct mass of mesoderm cells, and the secondary junction of the end-sac with the ecto-dermal kidney and duct. On the other hand, Grobben figures the end-sac cells as forming the upper extremity of the simple invagination in the early development of the gland, just as I have figured these same cells in my drawing of the youngest stage of the Squilla gland—there is no separate origin of the end-sac here—and Boutchinsky merely asserts that the deep end of the ectodermal invagination becomes surrounded by mesoderm cells—a statement which may have resulted from a mistaken conception of the origin of the "mesoderm" cells. Further, Waite's supplementary argument for the separate origin of the end-sac based upon the entirely distinct cha-racter of the cells of the end-sac as compared with those of the kidney counts for little ; my description of the differences of the kidney and duct-cells and their abrupt distinctness (no transitional kinds of cell existing) in the gland of Squilla might equally well be employed to suggest that the kidney and duct regions are of separate origin. In view, then, of the scanty and conflicting evidence on the subject, we are not in a position to draw conclusions respecting the ectodermal or mesodermal origin of the end-sac, still less to discuss the homologies either of the various parts of the antennal and maxillary glands or of the glands as a whole. Future inquiry can alone decide whether or not these Crustacean leg-glands (rudiments of which, it must be remembered, have been found in connection with the maxillipedes [15] and other thoracic limbs in addition to the second antenna and second maxilla) are to be compared with the ectodermal crural glands rather than with the mesodermal excretory organs of Peripatus as heretofore.

II. Notes on some other Features of the Internal Anatomy of Squilla.

The Communications between the Pyloric Digestive Gland ("Liver") and the Gut.

Johannes Müller (**29**) in 1830 made the statement that the so-called " liver " possessed numerous pairs of segmentally arranged openings into the alimentary tract, and later authors (e. g. Duvernoy, 1836 [**26**], Milne Edwards, 1859 [**27**], Gerstaecker, in Bronn's ' Thierreich,' 1889 [**31**] and others) have either accepted this statement on Müller's authority or have supposed that their reinvestigations have confirmed Müller. However, in 1901 Orlandi (**30**) to a large extent corrected this erroneous idea, and stated that the two halves of the " liver " solely opened into the gut by a single median opening into the dorsal side of the pyloric part of the stomach, and this statement has appeared in most recent English text-books, e. g. in Calman's " Crustacea " in Lankester's ' A Treatise on Zoology ' and Sedgwick's ' Student's Text-book of Zoology.' It must be pointed out, however, that Fritz Müller (**28**) in 1863 clearly described and figured a single pair of " liver " ducts opening into the sides of the stomach in an Erichthus larva, and this fact would by itself lead us to suppose that the adult condition is similar to that of the larva.

A further correction concerning this much misinterpreted and, indeed, trivial point of fact has now to be made in the present paper. Orlandi was correct in stating that each half of the " liver " only possesses a single duct opening into the pyloric region, but quite wrong in stating that the two ducts unite together and open by a single mid-dorsal aperture. Examination of complete series of transverse and longitudinal sections through the adult Squilla desmarestii, Risso, shows that each duct opens independently into the side of the pylorus by a large triangle-shaped aperture (the apex of the isoceles triangle being anterior and the vertical base posterior), and so conforms to the arrangement found in most Crustacea. In

front of these lateral openings of the pyloric gland into the stomach, the gland is produced into two blind diverticula which extend forwards as far as the base of the second

TEXT-FIG. 9.
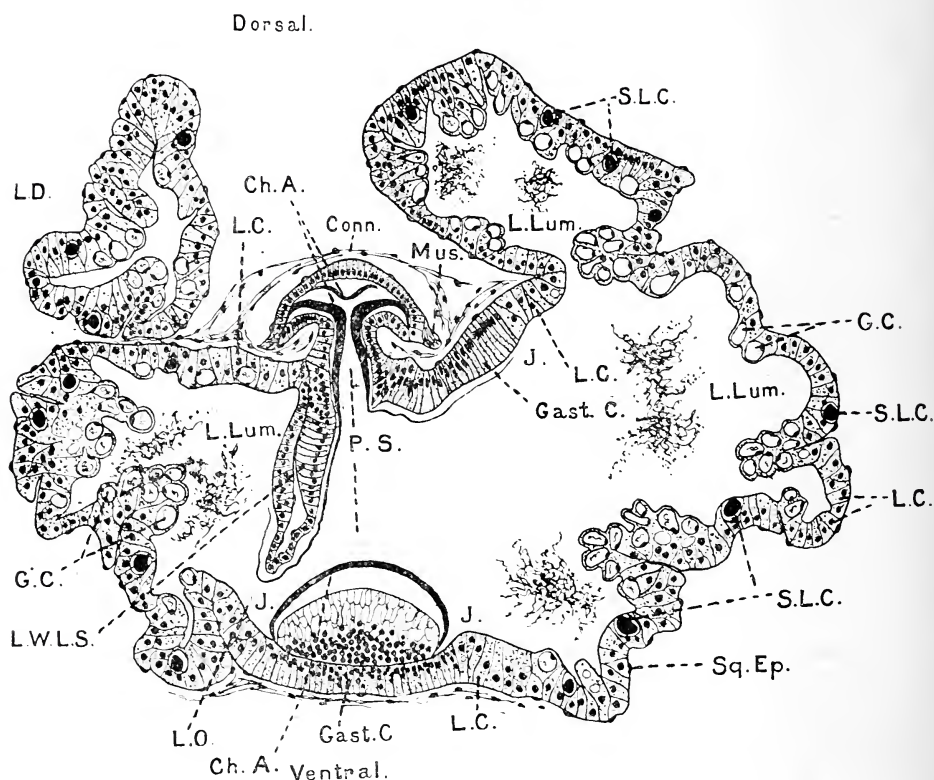
Transverse section across pyloric region of stomach, showing the two lateral openings of the pyloric digestive gland (explained in the text). *L. O.* Anterior extremity of left opening of gland into stomach. *Gast. C.* Gastric cells. *L. Lum.* Lumen of digestive gland. *L. D.* Small dorsal diverticulum of gland. *P. S.* Cavity of pyloric stomach. *Ch. A.* Chitinous lining of stomach. *S. L. C.* Small cells. *G. C.* "Goblet" cells. (× 173.) For other reference letters see p. 429.

thoracic limb. Each of these anterior diverticula is joined dorsally by a smaller diverticulum (*L. D.* in Text-fig. 9) at the level of the posterior end of the gland opening into the stomach.

Posteriorly to this opening the gland extends back on each side as a wide sac in close contact with the narrow intestine (which is thus effectively hidden from view), and gives off numerous lateral diverticula in its course. These diverticula are somewhat narrow proximally but dilate and branch distally; in the sixth abdominal segment they extend into the basal joints of the appendages borne on that somite, and they occupy the greater part of the cavity of the telson. Text-fig. 9 represents on its left side a section passing through the anterior extremity of the opening of the pyloric gland into the stomach, which is thus seen to be narrow and ventro-lateral; the right side of the same figure passes through the posterior extremity of the opening, which is thus evidently wide and lateral. Sections also show that the whole of the gland is enveloped in a large blood-sinus containing much blood.

The cells of the digestive glands are of several kinds, the most noticeable being the ordinary granular cells (*L. C.*), cells wedged in between the ordinary cells and containing large vacuoles (*G. C.*; these vacuoles burst into the gland cavity) and small cells with very large darkly-staining nuclei (*S. L. C.*).

The Rectal Glands.

The rectal glands are found in both sexes underlying the ramifications of the " liver " in the telson. They are two in number, are large, with spacious lumina, and open in the adult as in the larva (see Claus [5]), laterally at the posterior end of the rectum. In addition to these rectal glands there are present some small accessory tubules opening into the gut in the same region, which apparently bear some resemblance in structure to the urinary tubes of Amphipods.

The mesenteron is very long in Squilla and extends from the stomach to the region of the anus, where the cuticle of the proctodæum becomes inturned for only a short distance.

The Nauplius Eye.

The Nauplius eye persists in the adult Squilla, and can easily be seen under a low-power binocular as a small patch of black pigment lying towards the ventral side of the head between the bases of the paired stalked eyes. The pigmented eye apparently contains in the specimens examined either two or three ocelli (probably three, since this is the number found in most Crustacea), and is connected by a single slender nerve to the brain.

Appendix.

Methods of Preparation of Material.

The adult specimens of Squilla desmarestii were fixed for me at Naples in Hermann's fluid, in Zenker's fluid, in corrosive-acetic and in hot absolute alcohol. On receiving them I carefully decalcified certain specimens in a mixture of nitric acid (over 5 per cent.) in alcohol (the liquid constantly renewed) for three or four weeks. They were then embedded in hard (60° C.) wax and cut into sections $10\,\mu$ thick. Despite the thickness of the cuticle, complete series of transverse and longitudinal vertical sections were obtained of three entire animals. Complete series of transverse and longitudinal sections were also made of many larvæ of the sizes mentioned in the above description. All these sections were double-stained on the slide in Ehrlich's hæmatoxylin (twenty-four hours), followed by picro-indigo-carmine. (Add one part saturated solution of picric acid in 90° alcohol to two parts saturated solution of Grübler's indigo-carmine in 70 per cent. alcohol, and dilute this stain with equal bulk of 70 per cent. alcohol; for mode of using this stain see my paper on " Fish Gas Glands," ' Proc. Zool. Soc. Lond.,' 1911.) Sections of the Hermann's fluid material were found to be best preserved.

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REFERENCE LETTERS OF FIGURES (PLATE AND TEXT).

A. 1. Antennula. *A.* 2. Antenna. *Bl.* Bladder. *B. S.* Blood-space
(hæmocœle). *C.* Carapace. *C. D.* Duct-cells. *Cv. A.* Cephalic aorta.
Ch. A. Chitinous armature of stomach. *Conn.* Connective-tissue strands.
C. P. Col. Connections between columnar cells and "plugs" in cuticle.
C. Str. Striations in cytoplasm of kidney-cells. *Cut.* Cuticle. *D. Mx. Gl.*
Duct of maxillary gland. *Ds.* Dorsal surface of gland. *D. Sq. Ep.*
Double layer of squamous epithelium. *E.* Eye. *End. S.* End-sac. *End.
S. C.* End-sac cells. *Ep.* Epipodite. *Ep.* 2. Epipodite of second thoracic
appendage. *Ex.* Scale-like exopodite of antenna. *Exc.* Excretion
material. *F. C.* Free carapace. *Gast. C.* Gastric cells. *G. C.* Goblet-
cells. *G. D.* Lateral diverticulum of duct. *Gl.* Glands ("drüsenzellen").
Gr. Groove on side of gland lodging body muscle. *Gru.* Granules. *H.M.*
Hæmocœle. *Ht.* Heart. *Hyp.* Hypodermis. *I. C. D.* Invagination of
duct-cells. *I. M.* Invagination of kidney wall. *I. P.* Invagination of
partition wall separating end-sac and kidney. *I. Sq. Ep.* Inner layer of
squamous epithelium. *J.* Junction of gastric and hepatic cells. *L.* Pyloric
digestive gland ("liver"). *L. C.* "Liver" cells. *L. D.* "Liver" diverti-
culum. *L. Lum.* "Liver" lumen. *L. O.* "Liver" opening into stomach.
Lum. Lumen—excretory. *L. W. L. S.* Apposition of "liver" and pyloric
stomach on left side. *M. C.* Kidney-cells. *M. Mx. Gl.* Kidney portion
of gland. *Mx.* Maxilla. *Mx. Gl.* Maxillary gland. *Mxl.* Maxillula.
Mus. Muscles. *Mus. Lon.* Longitudinal muscles. *Mus. Tr.* Transverse
muscles. *Nc.* Nerve cord. *Op.* Opening of maxillary gland on papilla.
O. Sq. P. Outer layer of squamous epithelium. *P. C.* Protoplasmic
processes from end-sac cells traversing blood-space. *P. Col.* Mass of
columnar epithelium at opening of maxillary gland-duct. *P. S.* Pyloric
portion of stomach. *R.* Rostrum. *S. L. C.* Small special "liver"
cells (resembling oxyntic cells). *Str. B.* Striated border of kidney-cells.
Sp. Ep. Squamous epithelium. *Th.* 1, *Th.* 2, *Th.* 3 . . . *Th.* 7. Thoracic
appendages. *U. Th. G.* United ganglia supplying the mandibles and
the first five thoracic segments and bearing the circum-œsophageal
connectives anteriorly. *V.* Vacuole. *W. Bl.* Wall of bladder.

EXPLANATION OF PLATE 28,

Illustrating Mr. W. N. F. Woodland's paper, "On the Maxillary Glands and some other Features in the Internal Anatomy of Squilla."

[All figures of Squilla desmarestii, and all drawn by means of the camera lucida.]

Fig. 1.—Anterior end of Squilla, dissected to show the maxillary glands (*Mx. Gl.*) in situ. The carapace has been cut away dorsally over a large area, and the underlying hypodermis, some large oblique and longitudinal body muscles, the anterior cæca of the liver, and part of the gut and cephalic aorta have been also removed. (× 3.)

Fig. 2.—The maxillary gland (*Mx. Gl.*) removed from the body with the second maxilla and viewed laterally. Its external aperture (*Op.*) on the papilla (situated on the outer or convex side of the limb), the bladder (*Bl.*) and the mass of the gland can be seen. Most of the muscles entering the limb have been removed, and two are represented in outline by dotted lines. *Gl.*, small gland-cells. (× 6.)

Fig. 3.—Transverse section across the left side of Squilla in the region of the maxillary gland. As mentioned in the text, the details of the gland structure are shown on an immensely magnified scale in order to make apparent the general construction of the gland; in reality the cell-layers of the gland are much smaller and much more numerous. *M. Mx. Gl.* General (kidney) mass of gland. *D. Mx. Gl.* Duct of maxillary gland. *Bl.* Bladder portion of gland. *Op.* Opening of maxillary gland on papilla. *P. Col.* "Plug" of columnar epithelium at opening of duct (see Text-fig. 8). (× 20.)

On a Remarkable New Type of Protistan Parasite.

By

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With Plates 29 and 30 and 2 Text-figures.

In the course of a study of the flagellates, which develope in simple cultures of goat's dung, we were afforded recently the opportunity, by the kindness of Drs. E. H. Ross and J. W. Cropper, of examining the contents of the rumen and other parts of the digestive tract of a goat, with a view to ascertaining what flagellates occurred in the active condition in the goat. The results of our observations in this connection will be dealt with in another memoir. The object of this communication is to describe certain remarkable parasites which we found, which appear to be of a nature quite distinct from any other protist of which we are aware. These parasites occur under two characteristic forms, which we have distinguished respectively as crescents and ovals. We are strongly inclined to regard these two principal types as being different phases of one parasite. We have examined, up to the present, six goats, and in the rumen of each, either the crescents, or the ovals, or both forms have occurred in enormous numbers—far exceeding those of the ciliates or ordinary flagellates present. A comparison of the occurrence of the two forms is given in the following table :

Goat.	Crescents.	Ovals.
No. 1	Relatively infrequent	Abundant.
No. 2	Very abundant	Very abundant.
No. 3	Very abundant	Doubtful if present.
No. 4 (first time of examination)	Very scanty	Very abundant.
No. 4 (second time).	Much more frequent	Much less numerous.
No. 4 (third time) .	Numerous	Abundant.
No. 5 (first time) .	Present (see Note)	Present (see Note).
No. 5 (second time).	Fairly numerous	Numerous.
No. 6	Abundant	Very abundant.

Note.—The first three goats and the last one were killed, but in the case of the remaining two some of the fluid contents of the rumen were obtained by means of a stomach-tube. In the first examination of No. 5, only an extremely small quantity of material was obtained— scarcely any in fact—and this observation afforded no precise indication of the numbers of the parasite present.

Our attention was first directed particularly to the crescents because of their vigorous movements, and the fact that, in individuals which were moving but little or else were at rest, a single, conspicuous flagellum could be seen to be attached to the concave side of the parasite.[1] On account of this characteristic appearance we have given to this form the new generic name, Selenomastix. Before describing the parasite, however, we should point out that the crescents, at all events, have been undoubtedly observed before, for in the existing literature we are aware of two references which relate to this organism; but neither of them furnishes any true indication of its peculiar characters. The first record occurs in a short note by Certes ('Bull. Soc. Zool. France,' vol. xiv, 1889, p. 70), on the micro-organisms in the rumen of ruminants. This author observed, associated with the ciliates, a flagellate in the form of a cresent, which assumed at times an S-shape, and had its flagellum inserted at the middle

[1] We may add that the flagellum was actually observed first by Dr. E. H. Ross, in a preparation which he stained rapidly by the jelly-method, on being made acquainted with our discovery of the parasite.

of the incurved part of the body. The size is given as 8–9 μ long by 2–3 μ wide. Certes proposed the name A n c y r o m o n a s r u m i n a n t i u m for the parasite, although he recognised that there was very considerable difference between the new organism and the known species of A n c y r o m o n a s. (As will be seen from the subsequent account, the new parasite has nothing whatever to do with A n c y r o m o n a s.) Certes goes on to say that he found also in the rumen S a r c i n æ, the predominating forms being ovoid, hyaline and small, about 8–10 μ by 2–3 μ. The smallest forms showed sometimes the commencement of budding,. and in consequence might be associated with yeasts. Others, the great majority in fact, multiplied by fission. The ovals which we have found have certainly nothing to do either with yeasts or S a r c i n æ. It seems very probable, however, that they are the same thing as the predominating ovoids of Certes, which he erroneously connected with the smaller, budding organisms; these latter may have been of the nature of S a r c i n æ. The second reference is a brief note by Kerandel, in a paper on hæmatozoa observed in the Congo (' Bull. Soc. Path. exot.,' vol. ii, 1909, p. 208), to the effect that he had observed from the œsophagus of an antelope (C e p h a l o p h u s s p.) bodies similar to those previously noted by Certes. He refers to the conspicuous cilia inserted in the middle of the concave side of the body (cf. below, p. 440). Neither author gave any figures of the parasites, but it is apparent that both were dealing with the same creature which is here described. Hence, the new parasite must bear the name S e l e n o - m a s t i x r u m i n a n t i u m (Certes).

The rumen is undoubtedly the principal habitat of S e l e n o m a s t i x. A small drop of the fluid contents taken from any part of this bulky organ (in the case of killed goats) has always been full of one or other form of the parasite. The crescents have been found also, in sparing numbers, in the rumen of a sheep. Hence this creature appears to be a common parasite of ruminants, and, at any rate, in the goat is very abundant. The parasites also occur in small numbers

in the reticulum, and a few have been seen in the true
stomach, but they have never been observed in the intestine,
cæcum or rectum. In the last-named region of the alimentary
canal, cysts and spores of one kind and another occur, the
determination of which is a very puzzling matter, and it is
quite possible that some one of these represents a resistant
phase of the parasites ; but we have not succeeded in recog-
nising anything corresponding to the characteristic ovals.
Certainly no crescents are to be found, nor have they
developed in any of the many cultures which we have made
from the fæces, both of goats which we know to have been
infected and of others which in all likelihood also were. In
this feature Selenomastix agrees with the Ciliates and the
ordinary Flagellates which occur in an active phase in the
rumen. Like these, further, this new parasite appears to be
purely an inhabitant of the fluid contents of the rumen. We
have carefully examined the wall, both externally and inter-
nally, for any indication of cysts, large or small, but none have
been visible. (None of the goats examined have shown any
signs of the " cysts of Gilruth.")

We will first describe the appearance, behaviour and
structure of the crescents, and can then readily compare the
corresponding features in the ovals. Seen living and freshly
removed from the rumen, either with or without the addition
of a small drop of water or normal saline, all the crescents
have a very similar and characteristic appearance, but show
a considerable range of variation in size. The body has a
uniform, homogeneous, dull-looking appearance. We have
never observed the crescents alter in shape at all; they
are certainly not amœboid or " metabolic," and we have never
seen them assume an S-shaped form, as mentioned by Certes
(cf. below, p. 439). Many, if not most, of the crescents
possess a definite envelope, as is seen from stained prepara-
tions, but it does not stand out at all in life (contrast the
ovals, below). No granules of any kind, or vacuoles, are
noticeable in the protoplasm.

If examined as soon as possible after removal from the

rumen, a large proportion of the crescents are usually
actively motile.   As already mentioned, Selenomastix
possesses a single,[1] relatively large flagellum, which appears
to be inserted in the body, as a rule, about the middle of the
concave side (Text-figs. A–E).   The movements are very
varied.   We have distinguished the following kinds, but

TEXT-FIGS. A—G.
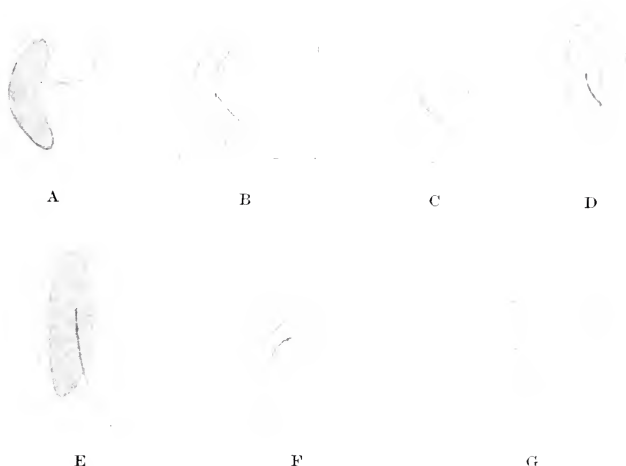
Crescents drawn either living or after fixation with osmic acid
   vapour.   A–C seen from the side; D and E showing the origin
   of flagellum from concave face; F and G two different stages
   in division.

though we have studied them very closely we are in some
cases not quite certain how they are caused. (1) The
flagellum is directed behind and undoubtedly acts as a
pulsellum, causing forward progression of the body, in a
rather irregular, zig-zag, but not spiral manner.   We have
not observed a definite reversal of the direction of this
movement, i. e. in the opposite sense, the other end of the
creature suddenly leading the way.   This appears to be the

[1] Many of the crescents have two flagella, but these are individuals
undergoing division (Text-figs. F and G).

least common type of movement, but we regard it as very important, since it provides a distinct indication of antero-posterior polarity. (2) The body oscillates to and fro, or turns round completely, one and a half times, or even twice, in the plane of its long axis, i. e. turning a somersault as it were ; no forward progression is effected. This movement may be quite rapid. When it can be seen, the flagellum stands out well from the body ; it may cause this movement, but we feel undecided, for we have noticed that when a crescent is becoming a little sluggish and the oscillations take place at longer intervals and not so rapidly, a premonitory tremble of the body occurs the instant before the movement. (3) Some parasites, which are moving only at intervals, can be seen to turn slowly from one side to another, this time around the long axis. In these cases the flagellum appears to be quite passive, and to turn over after the body. We certainly consider this movement is caused by the body and not by the flagellum. Moreover, now and again a crescent can be seen oscillating more or less, when it is impossible to see a flagellum in connection with it, and though of course it is sometimes difficult to make out the flagellum, nevertheless, with the aid of good lenses and critical illumination, this can usually be clearly seen ; further, crescents without a flagellum do undoubtedly occur. Hence we feel persuaded that move-ments caused by the body alone and not by the flagellum do take place (c f. also the ovals).

The movements of most of the crescents cease very soon after being removed from the rumen—surprisingly soon in some cases. After about an hour few are still to be found active, and their movements have become irregular and spasmodic. Even in a quantity of the fluid contents kept in a warm water-bath, at from 30°–35° C., in three or four hours the parasites were all motionless. Crescents may have short resting intervals and may then become actively motile again ; but when all or nearly all the crescents in a particular area of the drop under examination remain motionless, they may be regarded, we think, as being dead. In the rumen-contents

just referred to, though they were frequently examined during two days, we never found any active parasites after about four hours. The third goat examined furnished a remarkable illustration of this point. It was killed, and quantities of the rumen contents, taken from different parts, were put into two small dishes, which had been previously warmed. Not more than a quarter of an hour elapsed before these were examined, but although the crescents were extremely abundant, not one of them was active, though the flagellum could be made out in many. A warmed pipette was then taken and a fresh quantity of the contents obtained and immediately looked at; in the cover-slip preparations made here and there were areas of active parasites, though in other places they were motionless.

We consider that, in general, it is the lowering of the temperature which renders the parasites motionless, though we have come across exceptions. Thus, on one occasion, in a cover-slip preparation which had been kept at about 30° C. for twenty-four hours, a few parasites were still feebly motile. Again, in an endeavour to cultivate the parasites on agar plates, we have found two or three individuals still motile five days after being removed from the goat. In this character of extreme susceptibility to change of environment, Seleno-mastix agrees with the peculiar Ciliates and the Flagellates also present in the rumen; the Heterotrichous forms (Ento-dinium, Ophryoscolex) are even more quickly rendered motionless—indeed, frequently one can no longer find an individual still active—while the Flagellates are apparently just about as susceptible as Selenomastix, remaining active for two or three hours. On the other hand, the motile bacteria which occur (bacilli, spirillar forms) remain active for a much longer time.

We may add here that no development of the parasites occurs when "cultivated" outside the body, so far as we have been able to ascertain. We have tried simple agar plates, varying the strength and consistency of the medium. There is no further multiplication or apparent increase in number

either of the crescents or of the ovals. Ovals can be recognised longer than the crescents and are probably more resistant (cf. below); but by the end of eight days the medium is so overrun by bacterial and fungal growths that nothing else can be made out. Dr. Ledingham, of the bacteriological department here, kindly had cultures of the parasites made for us on different media, and he also informed us that no development took place.

Structure.—Selenomastix ruminantium presents some highly remarkable features in its morphology and minute structure. The usual and typical form is slightly crescentic (figs. 1, 5, 7, 18); in the larger individuals it is often very like a banana (figs. 25, 26). The parasite is never sickle or S-shaped; and this is true however big and long the crescents may be. Further, in the vast majority of parasites, if not in all, the curve of the crescent lies in one plane, *i.e.* it is not spiral like that of a spirillum. Viewed in this plane the parasites have the appearance of considerably elongated ovals (figs. 8, 9). Nevertheless, now and again, but very rarely, one gets the impression of the slightest possible twist in the axis of the parasite; thus in fig. 21 and to a less extent in Text-fig. E, there is an indication of one end of the body pointing rather in the opposite direction to the other. We have looked particularly for indications of a spiral character of the organism in life, and this appearance, which we have observed in only very few individuals, is the only one we have obtained. Moreover, we do not feel at all certain that this appearance corresponds to a permanent twist, however slight, in the body. For we have noticed, in watching certain individuals progressing forwards—sometimes, too, individuals in which no flagellum could be made out—that the hinder part of the body moves slightly to and fro, laterally, in a zig-zag manner, distinctly more so than does the front part, which is kept fairly steady; this may perhaps be caused by a slight voluntary twisting of the hinder end of the body, first in one sense and then in the opposite one, this movement serving to propel the body (cf.

Certes' remarks, above, though there is never anything approaching an **S**-shape).

The concavity of the crescent may be only very slight (figs. 1, 14, 16), or may be practically absent (figs. 2, 4, 11, 12) ; small forms often appear thus. Individuals immediately resulting from division may be pyriform, differing from the ovals in having one end broader than the other (figs. 34, 35). We have not succeeded in finding an individual with both sides markedly convex, which at the same time possesses a flagellum ; in other words, we have not found a typical oval with a flagellum. The nearest approach to an oval shape is seen in figs. 4 and 11, and these individuals, though they still come in the category of crescents (for one side is practically straight), nevertheless closely resemble certain ovals. When seen more or less in the plane of the curve, the body of a crescent can be distinguished from that of an oval by the fact that its ends are narrower and more tapering (figs. 8, 9).

The flagellum is apparently always attached to the concave (or straight) side of the body, and in the majority of cases its point of insertion is about the middle of this side. But this point varies to a certain extent, particularly in the smaller forms, where the flagellum may arise much nearer to one end (figs. 1, 3, also 18) ; we have never found it, however, actually terminal in origin. It is possible that this variation in the point of attachment of the flagellum may be partly dependent upon the process of division. The question of the orientation of the body is one of much difficulty. If the middle point of insertion of the flagellum represents approximately the anterior end, then it is obvious that the body is greatly extended laterally. It is certain, however, that the parasite never progresses forwards in a direction at right angles to its long axis, i. e. broadside on, as it were. We have, in fact, no reason to suppose that this is the right view to take. The only clue to an orientation of the body is the indication we get from certain movements of progression of the parasites of an antero-posterior polarity ; in such cases

the end nearest to which the flagellum is inserted goes **first**
and may be regarded as anterior, the flagellum itself **being**
directed backwards.

As regards the dimensions of the parasites, crescents **of**
average medium size have a length of 9·5 to 11 μ and **a**
breadth of 2 to 3 μ (Text-figs. A–D and figs. 1–8, 11–16),
the length of the flagellum being about 8–9·5 μ. The **largest**
(single) individual we have observed (on a " wet-fixed " film)
is 12·5 μ long by 3·25 μ broad and the flagellum is 15 μ **long**
(fig. 10) ; on a " dry," Giesma smear, the largest crescent
found measures 13·5 μ in length by 3·75 μ in width,[1] the
flagellum being 12 μ long (fig. 77). The smallest crescent
observed, just in the act of separating after division (fig. 33),
is only 4·25 μ by 1·9 μ ; another small one (fig. 35) is 6·25 μ
long and rather stouter, being 2·5 μ broad. Between these
extremes all intermediate sizes occur.

The flagellum itself may be as long as 16 μ (fig. 2) or as
short as 7·5 μ (fig. 12) ; its length does not bear any **very**
close relation to the size of the parasite, the small individual
of fig. 5 having a very long flagellum, while the large **para-**
site of fig. 19 has a relatively short one. A remarkable fact
bearing upon the structure of the flagellum is brought out **by**
" dry " Giemsa-stained smears. In wet-fixed films the flagel-
lum does not apparently differ much from that of an ordinary
flagellate ; it has, perhaps, a thicker and stronger appearance
on the whole, though it usually thins out a little and becomes
more tapering towards the free end. On Giemsa smears, how-
ever, the flagellum is frequently seen to be more or less
broken up into separate bands or fibrils, often throughout the
greater part of its length (figs. 75–77) ; or else it has split
into two or three fibrils near the free end. This appearance
has certainly nothing to do with division, which is quite
different (cf. below) ; moreover, we have never seen it in

[1] Some of the parasites on Giemsa smears are possibly a little **too**
wide relatively, having been flattened out slightly in making the **prepa-**
ration : on the other hand, the parasites on wet-fixed films are probably
slightly (uniformly) contracted.

wet-fixed films.   Apparently the somewhat rougher treat-
ment of the parasites in making a Giemsa smear—perhaps the
drying—may cause the flagellum, in certain cases, to be
partially broken up into component fibrils.   This observation
is very interesting, because it points to the flagellum having
a structure rather different from that of most ordinary flagel-
lates, for, in the course of our work on the forms which crop
up in the fæcal cultures (e. g. Monas, Cercomonas,
Bodo), we have never observed such a splitting of the
flagellum, and we have made numerous Giemsa smears.[1]

It is apparent from wet-fixed preparations that a definite
membrane or envelope surrounds many, if not all, the
crescents.   It is curious that, within a short distance of one
another, parasites can be found, both single individuals and
forms undergoing division, which show indications of this
envelope to a very varying extent.   Thus it may be seen,
standing off from the general protoplasm of the body, only at
one end (figs. 23, 35, 36) ; or at both ends (figs. 6, 16) ; or
along one side (fig. 2) ; or nearly all round the body (figs. 3, 19,
27).   In others, again, it is not discernible at all (cf. figs. 1, 4,
7).   We are uncertain whether these different appearances
represent the actual condition in life, or whether they are to
some extent due to the body-protoplasm having undergone a
certain amount of shrinkage away from the envelope in the
wet-fixation, more especially in the direction of length.   As
mentioned above, no envelope, distinct from the general
body-substance, can be made out in the living crescents, nor
is it obvious, as a rule, in the parasites on Giemsa smears.   A
point to notice is that the envelope never stands off from the
general protoplasm at the point where the flagellum is
attached; this indicates that the latter organella is not
merely a development from the membrane, but originates

[1] This splitting is never seen in the living parasites, but it is in-
teresting to note that we have observed a somewhat similar splitting
of the flagellum (in life) in a " true " flagellate occurring in the rumen
(perhaps a Sphæromonas), which possesses a long, thick, curved
flagellum.

from the general protoplasm. We may say here that there
is not the faintest hint of any groove or depression around
the body of a crescent at the point where the flagellum starts.

Not the least remarkable feature of Selenomastix is its
cytology. The best stain is undoubtedly iron-hæmatoxylin.
Delafield's hæmatoxylin shows just the same minute structure,
but it has the drawback that the flagellum is usually very
faintly stained and often cannot be made out, and the same
remark applies to carmine stains. Giemsa's stain is of con-
siderable use in many respects, but not of much service in
bringing out the details of the internal structure of the body,
except where the chromatin is in the form of one or two
prominent masses. The general protoplasm nearly always
stains uniformly and homogeneously, sometimes lighter and
sometimes darker, according to the degree of extraction. It
never shows either granules of any kind or vacuoles.

There is no properly constituted nucleus, either of the
usual karyosomatic type seen in the flagellates, or of any
other type with which we are acquainted. Nevertheless,
chromatin is undoubtedly present in greater or less quantity,
occupying a peculiar but characteristic position. The prin-
cipal situation of the chromatinic substance is at the periphery
of the body. In the condition in which the parasite has
apparently the least amount of chromatin, this constitutes a
very narrow layer or zone, extending all over the surface of
the body and appearing in optical section as a definite border,
staining blacker and more intensely than the cytoplasm
(figs. 3, 5, 6). More generally, however, this layer shows
distinct thickenings, which may take the form either of
numerous fine, small granules, appearing as little more than
dots (figs. 1, 4, 8); or of few or several larger more con-
spicuous grains or small masses (figs. 9, 11, 10, 17); or, finally,
of a few (usually one or two) quite large, dark-staining masses
(figs. 12–16, 26). The granules and masses project inwards,
one edge always being at the surface of the body, and we are
inclined to consider them as having developed from the basal,
peripheral zone or layer. Now and again these dark masses

form thick half-hoops or rings, partially encircling the body
(figs. 18, 19). In large individuals they may occur together
with conspicuous granules in the peripheral zone (fig. 25),
but as a rule in the smaller forms, when a prominent
chromatinic mass is present, the peripheral zone appears to
contain very little chromatin.

Division.—Whenever the crescents have occurred in
numbers we have found division proceeding actively. Divi-
sion of the parasites always takes place by means of equal
binary fission. We have never seen the slightest indication
of unequal fission, or of anything in the nature of budding.
So far as we have been able to ascertain, binary fission
appears to be the only form of multiplication in Selenomastix.
The division always takes place in a plane at right angles to
the long axis of the body, i. e. it is transverse to it. Division
does not stand, apparently, in any definite relation to the
size of the parasite ; that is to say, not only large individuals
divide, but intermediate-sized ones and also quite small forms.
Neither does the condition in which the chromatinic substance
is present appear to determine fission, for individuals can be
found undergoing division in which the chromatin is prac-
tically in any of the states described above (cf. figs. given
of dividing forms).

In the great majority of cases, though not by any means
always, the fission is initiated by the splitting of the flagellum
along the greater part of its length. This is shown clearly
in figs. 20, 23. There is no question of this appearance being
merely a fraying-out of the flagellum into fibrils, such as was
referred to above. For one thing, the instances figured (and
others observed) are on wet-fixed films, in which the fraying-
out is never found. Again, when the flagellum shows a
frayed-out appearance, it is either the middle portion or else
the free distal end which is split into fibrils of varying
thickness ; in the true splitting of the flagellum, leading to
division, the basal part divides first of all into two daughter-
flagella of equal thickness, the proximal, attached ends first
separating. In the figures mentioned, the splitting has not

yet proceeded along the entire length, and the two daughter-flagella are still united into one distally. Probably the parent-flagellum splits nearly throughout its length, for the two daughter-flagella are usually approximately equal (figs. 24–28) ; it is exceptional to find a dividing individual in which there is as great a difference between the length of the separated flagella as in that of fig. 31. We have been considerably exercised in regard to the question whether there is a basal granule in connection with the flagellum. We are rather inclined to think that there may be such a granule, but we cannot say with certainty. There is frequently a definite granule exactly at the point where the flagellum originates (figs. 11, 13, 21 and 22), but owing to the peripheral situation of the chromatinic zone, it is possible, of course, that such granule is a chromatin grain. Nevertheless, in such a case as is shown in fig. 20, where the chromatinic zone is very feebly developed, but where there is a very distinct granule-like thickening at the basal end of each of the daughter-flagella, definite basal granules are certainly suggested.

As regards the division of the chromatinic substance, we have found nothing to indicate that there is any pronounced attempt at equal distribution between the two daughter-individuals. Apparently, the chromatic substance which happens to be in either half, prior to division, goes to that daughter-individual (cf. figs. 24 and 25). However, the most usual condition in which the chromatin occurs in dividing individuals is that of a number of small granules fairly uniformly distributed around the periphery (figs. 27, 28), and therefore there may be really a nearer approach to equalisation than is obvious owing to the absence of a definite nucleus. The last act in the process is the constriction of the general body-substance into two halves ; this always takes place exactly in the middle of the long axis.

In some cases fission of the body undoubtedly occurs before the flagellum has split (figs. 32, 30, 29 show different stages in such a process). Whether the daughter-individual which thus lacks a flagellum is able to develop one we cannot say,

but crescents which do not possess a flagellum certainly occur (figs. 36, 37). We are inclined to think that such forms may become ovals, which we have next to consider.

The Ovals.—In their general appearance the ovals resemble the crescents. Their average size also is quite comparable; they are somewhat shorter, but distinctly more bulky. Some of them are seen to be considerably elongated, but these are individuals either about to divide or in the act of dividing. Apart from their shape the essential point of difference from the crescents is that the ovals entirely lack the characteristic flagellum. Nevertheless the ovals are undoubtedly capable of movement, and this fact was brought home to us in a surprising manner. When the fourth goat was examined for the first time an enormous number of active ovals were found, while the crescents were extremely scanty —far fewer in number than on any other occasion. The great majority of the ovals were in motion, the movement being one of progression, in a slightly zig-zag manner, but no sudden reversal of the direction of movement was noticed. At each of the subsequent examinations of the same goat, when the ovals have been relatively fewer and the crescents more numerous, most of the ovals have been quite still (although there were active crescents in the same preparations). Here and there, however, an oval would be seen zig-zagging to and fro slightly and spasmodically, scarcely progressing at all. And this has been the case in most of the other goats examined; nearly all the ovals were motionless. We have not observed any rotation of the ovals on their own axis, such as is commonly seen in the crescents. The remarkable activity of the ovals on the particular occasion referred to soon subsided, and after about a couple of hours they were all still. In the last goat examined, however, many of the ovals, as well as the crescents, were active, and we were able to observe a distinct indication of antero-posterior polarity in their case also. Now and again an oval steadily progressing would come against an obstacle. When this occurred the oval did not move away in the opposite sense, but turned

quite round and went off in another direction, the same end still being in front.

We had some of these active ovals specially stained for us by one of the principal methods in use (de Rossi's) for showing up the flagella of bacteria, but with no result whatever. We have ourselves tried this method and also Pitfield's method, with equally negative results. In short, in none of our preparations, however stained, have we ever seen a flagellum or tuft of flagella in connection with an oval; and we feel convinced that the ovals do not possess flagella of any kind. We are supported in this view by two points: (1) The close agreement in minute structure shown by many of the ovals and crescents, and the fact that the flagellum of the latter is a well-developed structure, readily visible; and (2) the conviction we have gained that the crescents themselves are capable of movement by other means than their flagellum.[1]

While many of the ovals have the same homogeneous appearance in life as the crescents, in some the membrane or envelope stands out distinctly, being separated from the general body-substance by a narrow, clear area (Text-figs. H, L and M). In the majority of the ovals, the envelope appears to be more prominent and more distinct from the body than in the crescents, and this is borne out by the study of stained preparations. Probably it is a firmer, more resistant structure in the ovals.

Structure.—The ovals are rarely, if ever, spherical; the nearest approach to a spherical shape is seen in forms immediately resulting from division (figs. 44, 55, 57), and even in these one diameter is usually greater than the other. On the other hand, they are rarely sufficiently long in proportion to their width, and the two longer sides sufficiently straight and parallel, for them to be regarded as rod-like; here, again, the nearest approach to such an appearance is shown by those forms about to divide (fig. 56). Undoubtedly the oval

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[1] It may be added, perhaps, that neither do the crescents show any flagella of the bacterial type when stained by the above-mentioned special methods.

shape is typical of this phase of the parasite. The average size varies from $7-9\frac{1}{2}\mu$ in length, by $3\frac{1}{2}-5\mu$ in width; individuals with dimensions less than these occur, but larger ones are nearly always in the act of dividing; the individual of fig. 48 is about the largest single oval found.

In the case of the ovals, the minute structure can be made out satisfactorily only in wet-fixed preparations, stained by hæmatoxylin; in Giemsa smears, the ovals—especially the large ones—stain much more intensely than the crescents, and usually appear rather blotchy, the stain being deposited to a greater extent either in, or immediately beneath, the (thicker) envelope. Hence, only two or three examples

TEXT-FIGS. H–M.

H J K L M

Ovals drawn either living or after fixation with osmic acid vapour. H, L and M show the envelope distinctly; in J and K it is not visible.

stained in this manner are figured, for the sake of comparison (figs. 83–87). The ovals show two types of minute structure, which, at first sight, appear quite different; we think, however, that they are connected by intermediate conditions. The first type of structure is practically identical with that of many crescents. There is just the same difference with regard to the distinctness of the envelope. In many individuals it stands off well from the general protoplasm at the two ends of the body (figs, 39, 40, 53); in others, though these are fewer in number, it is not apparent at all (figs. 41, 43, 45, 54). The protoplasm stains in the same uniform manner and shows neither granules nor vacuoles. The chromatinic substance is distributed in the same characteristic manner, constituting usually a zone of

fine granules closely arranged round the periphery (figs. 39–41), or, more rarely, comprising fewer, somewhat more prominent granules (figs. 45, 46, 49). We have not found any ovals with one or two large, deeply staining masses of chromatin such as are shown by some of the crescents. Further, the ovals divide in just the same way, by equal, transverse, binary fission (figs. 49–53). Whatever the crescents are, we think there can be no doubt that these ovals are, at any rate, a very similar type of thing (c f. especially figs. 6, 27 of crescents with figs. 40, 53 of ovals); the only essential point of difference is that the latter have no flagellum.

In the great majority of the ovals which show the second type of minute structure, the envelope projects markedly at both ends of the body (figs. 65–69), and now and again it stands off slightly also at the sides (figs. 64, 66, and fig. 87 on a Giemsa smear). The general protoplasm is usually sharply divided into two distinct zones, a central, lighter-staining region and a peripheral, more deeply staining area, which is usually wider at the two ends. The lighter staining, central area appears very similar to the general cytoplasm of the other ovals, and is, we consider, comparable to that. The darker-staining zone appears practically homogeneous, and does not contain, or is not composed of, the fine intensely staining granules characterising the chromatinic zone of the first type of ovals. The comparative extent of the central pale area and the surrounding darker region varies greatly in different individuals. In some the central area is small and the dark zone thick and broad (figs. 65, 67, 68); in others the paler area is much increased and the peripheral zone reduced to a narrow band (figs. 58, 59). Frequently, with this increase of the paler area, the dark-staining substance persists chiefly in the form of two caps, one at each end of the oval, connected only along the two sides of the oval by an extremely thin peripheral layer (figs. 60, 69). Lastly, in a small proportion of ovals, all the protoplasm appears to consist of the darker-staining substance (figs. 64, 70); these may be either small or fairly large.

We have not found such well-marked indications of division in ovals possessing a large area of dark-staining material as in those of the other type; but we are inclined to think that the same transverse binary fission occurs. A not uncommon feature in ovals of this type is the presence of two definite, intensely staining granules, one at the middle of each of the longer sides of the body (figs. 67, 69–71). Frequently these two granules are connected by a fine line, which is sometimes seen to follow the external contour of the body (fig. 70), when it probably represents a very slight annular constriction across it; but at other times the line can be traced with difficulty through the body (fig. 69). It seems probable that these appearances indicate transverse division, but we do not think it occurs to nearly the same extent in ovals showing this second condition of the internal structure.

We may now consider briefly the question of the connection of these different types of form with each other, and of their association together as different phases of one parasite. In the first place, ovals showing the second type of minute structure can be readily connected with those showing the first condition described, by a series of intermediate stages. All degrees in the thinning out of the darker staining area until it is little more than a narrow peripheral ring (as in fig. 59) can be found; and from such a stage to that shown by the individuals, for instance, of figs. 39 or 40 is a very slight transition. Another marked transition stage is seen in fig. 42, where the narrow peripheral, intensely staining zone is slightly thickened around one end; such a condition is manifestly closely connected with that showing a cap of dark-staining substance at each end (as in figs. 60, 61). It is a little difficult to know what interpretation to assign to this darker-staining part of the protoplasm, as found in the second type of oval. In the ovals with a well-marked, finely granular peripheral layer, or with more conspicuous granules, we consider that this zone comprises the chromatinic material of the cell, just as in the case of the crescents. Are we, then, to regard the more or less homogeneous, darker staining

region, when present, as representing chromatin or some allied substance diffused in an extremely fine condition throughout a relatively large area of the protoplasm?

Secondly, as regards the first type of ovals and the crescents, there are several reasons for concluding that these are only distinct phases of one parasite. There is the fact that, on all occasions save one, we have found the two forms associated, and possibly in the case of the third goat ovals may have been present, but were so scarce in comparison with the enormous number of crescents that we did not notice them. Important points of agreement between the two types as regards appearance, one manner of movement, structure, the occurrence of crescents without a flagellum, and so on, have been already dealt with. Lastly, in the case of some individuals, it is purely a matter of choice whether to regard them as bean-like crescents, or as bean-like ovals; thus the form drawn in fig. 38 is readily derivable, one may reasonably suppose, from an aflagellate crescent such as that of fig. 36, while on the other hand, between the parasite of fig. 46 and the oval of fig. 45 there is equally little difference.

Concluding, then, that the above-described different forms all belong to one parasite, it still remains a matter of uncertainty what is the order of transition between them, respectively, and how the different phases should be combined into one life-cycle; it appears very probable that the crescents can give rise to ovals; but we have no indications as to whether the ovals become crescents. Further, we are inclined to the view that the second type of ovals pass into the first type, rather than vice versâ.

The Nature and Affinities of Selenomastix ruminantium.—It will be apparent from the foregoing account that this new parasite does not fall readily into any of the known groups of organisms included under the designation Protista; in many respects it is an altogether new type of organism. On first seeing the living, active crescents, with their conspicuous, long flagellum, we naturally thought we had to deal with a new member of the flagellates, as, indeed,

was Certes' opinion originally. From a further study of Selenomastix, however, we feel at present very doubtful whether it is a true flagellate. Supposing for the moment that it is, the question of the orientation of the body is a very important one, because this determines, of course, the nature of the division. We have not the slightest indication that the middle of the concave side—approximately the point of insertion of the flagellum—represents the anterior end; on the contrary, such evidence of antero-posterior polarity as we have obtained points to this being in the direction of the longer axis of the body, both in the crescents and the ovals. Hence we must regard the division as transverse. Apart from the entire order of the Dinoflagellates, there are scarcely any Flagellates in which division is transverse. We can find no hint whatever of Dinoflagellate characters in Selenomastix. In a crescent which is not commencing to divide, there is neither a second flagellum nor any sign of an annular, transverse groove. It is equally difficult to see any indication of relationship among the Euflagellates. For one thing, the peculiar scattered or diffuse condition of the chromatinic substance is very different from the definite nucleus which is typical of flagellates. Another point which in our view weighs very much against the flagellate affinity of this new creature is the conviction we have that it is capable (either in the crescent or the oval phase) of moving by means of its body alone, somehow, independently of the flagellum, when this is present. We have next to search, therefore, among the vast assemblage of organisms collectively known as bacteria for a clue to the relationships of Selenomastix.[1]

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[1] So far as the ovals alone were concerned, we did not overlook their possible connection with some of the Saccharomycetes, such as Schizo-saccharomyces. Thanks to the kindness of Dr. Harden, of the Lister Institute, we have been able to compare the ovals with organisms of this group, and it was at once apparent that with them they have nothing whatever to do. Further, the ovals do not resemble in any way another yeast-like type of organism, namely Blastocystis, which has been lately described.

We have considered it useful to discuss the possible
relationship of Selenomastix to the flagellates, because not
only does our parasite differ in many respects from any
bacterial protist of which we have knowledge, but it also
appears to incline more to the Protozoa in one or two
important features. In the first place, the host of ordinary
bacteria may be at once dismissed from consideration. Dr.
Ledingham has kindly looked at the parasites and entirely
agrees with us in this opinion ; moreover, as above mentioned,
cultures made on various media were quite unsuccessful. The
most striking feature of Selenomastix, from a bacterial
point of view, namely, the presence of a large flagellum,
easily visible in life and by ordinary staining methods, is
only met with, so far as we are aware, in the case of one
or two very large spirillar forms and among certain " Sulphur-
Bacteria " (Rhabdomonas, Ophidomonas), of which a
good account has been given by Bütschli (' Arch. Protistenk.,'
vol. i, 1902, p. 41). The spirillar forms and Ophidomonas
have a flagellum at each end, Rhabdomonas a single,
terminal one. In these forms, too, the flagellum shows a
tendency to split up, in a somewhat similar manner, into
fibrils of varying thickness. These forms have also a well-
marked envelope (periplast), which stand off well from the
body in many cases ; that of Rhabdomonas is spirally
striated, a point which we have never seen in Seleno-
mastix.

It is possible that the origin of Selenomastix is to be
sought amongst this type of organism ; we have had it tested
for the presence of sulphur, however, with entirely negative
results. Moreover, Selenomastix certainly appears very
far removed from the spirillar type as generally recognised.
Taking first the points of agreement, there is, of course, the
transverse division and the absence of a definite, constituted
nucleus. Another feature which is somewhat against the
Protozoan character of our parasite is the peculiar homo-
geneous appearance of the protoplasm ; the ordinary true
flagellates, for instance, which occur in the rumen, look very

different, with their granular cytoplasm. In this respect
Selenomastix agrees with many bacteria, though it so
happens that spirilla, especially the larger ones, are often
distinctly granular and sometimes exhibit a chambered
structure, of which there is no sign in this new form.

The principal differences from the spirillar type are as
follows : There is no true spiral form of the body, or indica-
tion of spiral movement. In some individuals, however, there
is a hint of a twisting of the axis towards one end, which
may or may not represent a permanent condition. There are
no terminal flagella, but a single more or less median one.
There is distinct evidence of antero-posterior orientation.
On the other hand, we have never observed the reversal of
direction characteristic of spirilla. Another important dis-
tinction is that both the crescents and the ovals can move by
means of the body alone, resembling a Spirochæte, with
which, however, Selenomastix has assuredly nothing else
in common. Again, individuals certainly vary in width as
well as in length; broadly speaking, the larger individuals
are both longer and wider than the smaller ones; this is
apparent from our plates. Last, but not least, no spirilla of
any kind hitherto described, so far as we know, have any
phase connected with them comparable to the ovals of
Selenomastix.

With regard to the vexed question of plasmolysis, we are
inclined to think that this occurs, at any rate in the ovals.
In living preparations which have been made for some time
(whether diluted with a drop of normal saline solution or not),
a small proportion of the ovals show a vacuole, or space-like
appearance near one or both ends ; this is most probably due
to the shrinkage of the protoplasm away from the envelope.
When the parasites are placed in 5 per cent. or in 10 per
cent. salt solution, a somewhat larger proportion of the ovals
show this shrinkage appearance, and it is evident, also, here
and there in a few crescents. Many of the ovals, however,
and the great majority of the crescents—chiefly those, we
think, in which, if stained, the envelope would not stand off

in a marked manner—do not appear to be altered at all. They do not swell up, burst, or undergo any other obvious change. An interesting fact, moreover, which we noticed was that, when such preparations were looked at again the next morning, there did not appear to be as many ovals showing the contracted protoplasm as there were soon after the preparations were made. It seemed to us as if the proto-plasm must have expanded again in some individuals, which could not, therefore, have been dead.

Of one thing we are sure, namely, that Selenomastix does not undergo what the Germans term "Präparations-plasmolyse." This is evident from our figures, which give a fair assortment of the various appearances seen in the stained preparations. Lest it might be thought that the condition in which there are one or two deeply staining masses (regarded by us as chromatinic) in the cell, represents such an artifact, we may point out, first, that there is no larger proportion of such individuals on "dried" Giemsa smears than occurs in properly "wet-fixed" films, made as soon after removal from the rumen as possible; secondly, that every transition can be traced in the development of this phase where the chromatinic substance is compacted into few masses, through conditions where there are a varying number of smaller, but quite prominent granules; and finally, if it were an artifact, the ovals with the first type of minute structure, closely com-parable to that of the crescents, might be expected also to show it, which is never the case.

We have now, we think, considered exhaustively the possible directions in which to look for the origin and affinities of this remarkable parasite, so far as we have been able to do so from the facts we have learnt with regard to it up to the present. To sum up, it appears to be entirely unconnected with the Dinoflagellates; it may possibly be derived from some large spirillum, or from an Ophidomonas- or Rhab-domonas-like form, although we are very doubtful upon the point. For our own part, we are inclined to hazard the suggestion that if there is such a thing as a Pro-Protozoan or

Pro - Flagellate, Selenomastix ruminantium (Certes) represents such a Protist, because of the fact that it exhibits certain characters which are common to the flagellate Protozoa, but which are rarely or never possessed by bacteria.

SUMMARY.

(1) This paper describes a new type of parasitic Protist, to which we have given the name Selenomastix ruminantium (Certes). Its habitat is the rumen of Ruminants, especially that of the goat.

(2) The organism occurs in two chief forms—crescents and ovals. The crescents present a homogeneous, non-granular appearance, and possess a definite envelope; a single, large flagellum, conspicuous in life, arises from about the middle of the concavity of the crescent. The method of movement is variable; while the movement is sometimes effected by the flagellum, in other cases, perhaps more usually, it is produced by the body alone. In forward progression distinct anteroposterior polarity can be recognised. There is no properly constituted nucleus, the chromatin being present in the form of a peripheral layer, in which granules of varying size may occur, or there may be one or two large masses projecting into the cytoplasm. Division is by equal binary fission, transverse to the long axis.

(3) The ovals resemble the crescents in general, but they never possess a flagellum, although capable of active movement. They show two types of minute structure : (A) ovals in which the chromatinic substance occurs as a narrow, peripheral layer, with or without granules in it. This arrangement agrees closely with that found in the crescents. (B) Ovals in the protoplasm of which two zones can be distinguished , a central, lighter-staining zone, comparable to the cytoplasm of (A) and of the crescents, and a peripheral, darker area of variable extent. This latter may be chromatinic in nature.

(4) We suggest that the second type of oval gives rise to the first type, and also that the crescent may pass into the first type of oval by the loss of the flagellum. We have no indication whether the crescents may be developed from the ovals or not.

(5) Apparently the only Flagellates from which this organism could be derived are the Dinoflagellates, and, apart from the transverse division, there is no indication of any affinity with this group. Further, the nature of the " nucleus " and the capacity of moving by the body alone make it very doubtful if this parasite is a true protozoan.

(6) Selenomastix ruminantium differs in important respects from any known bacteria. It has no affinities with Schizo-saccharomycetes, with Blastocystis, nor with the Spirochætes. In certain characters it shows a resemblance to one or two large Spirillar forms, or to certain members of the Sulphur-Bacteria (e. g. Ophidomonas), but while its derivation is possibly to be sought in this direction, it is, nevertheless, very far removed from such forms. We may have in Selenomastix an example of a Pro-flagellate.

EXPLANATION OF PLATES 29 AND 30.

Illustrating the paper by Dr. H. M. Woodcock and Mr. G. Lapage, " On a Remarkable New Type of Protistan Parasite."

[All the figures are magnified 2000 times linear. We are indebted to Miss Rhodes for kindly drawing a few of them.]

Figs. 1–38.—All the figures are of crescents, and are from " wet-fixed " films, stained by iron-hæmatoxylin.

Figs. 1–19.—Single individuals, of various size, showing different conditions of the envelope and of the chromatinic substance.

Figs. 20–26.—Dividing individuals possessing two flagella, but in which the body does not yet show indications of fission. In figs. 20 and 23 the actual splitting of the flagellum is shown.

Figs. 27, 28, and 31.—Later stages of fission, in which the body is also dividing.

Figs. 29, 30, and 32.—Individuals in which the body is dividing, but of which the flagellum has remained single. One of the daughter-individuals will be aflagellate.

Fig. 33.—A small individual in the very last stage of division.

Figs. 34 and 35.—Small pyriform individuals, probably immediately resulting from fission.

Figs. 36 and 37.—Aflagellate crescents.

Fig. 38.—Bean-like crescent, which may be transitional to an oval.

Figs. 39-71 are of ovals, from "wet-fixed" films, stained by iron-hæmatoxylin.

Figs. 39-57.—Ovals with the first type of minute structure.

Figs. 39-48, 54.—Single individuals of various size.

Figs. 49-53, 55 and 57.—Individuals showing different stages of fission.

Figs. 58, 60-62, 64-68.—Single individuals showing the second type of minute structure.

Figs. 59, 61.—Individuals transitional between ovals of the first and second type.

Fig. 63.—Small dividing individual.

Figs. 69-71.—Ovals showing two definite granules at opposite sides, connected by a line or ring (see text). The individual of fig. 71 is apparently beginning to divide.

Figs. 72-82 are of crescents stained by Giemsa.

Figs. 72 and 73.—Single individuals; flagellum normal.

Fig. 74.—Dividing individual; flagella normal.

Figs. 75-77.—Individuals showing artificial fraying-out of the flagellum.

Fig. 78.—Smallest crescent found on a Giemsa smear.

Fig. 79.—Dividing individual, the upper flagellum of which shows indication of fraying-out.

Figs. 80-82.—Aflagellate crescents, showing one or two conspicuous chromatinic masses.

Figs. 83-87.—Ovals stained by Giemsa. In fig. 87 the envelope stands off markedly from the body and shows an annular line (cf. figs. 70 and 71).

Studies on Parasitic Protozoa.

II. (a) The Encystment of Rhizomastix gracilis Alexeieff; (b) Tetratrichomastix parisii n. sub-gen., n. sp.

By

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With Plates 31 and 32.

INTRODUCTION.

THE intestine of that common grub, the larval Tipula, contains a rich bacterial flora, at the expense of which there flourish a surprising number of protozoa. Among these are at least eight flagellates and two amœbæ.[1]

It is customary to speak of such an intestinal fauna as parasitic, a word which implies that these organisms are in some sort harmful to their host. A short study of the actively moving and actively feeding protozoa in the gut of the crane-fly larva suggests much rather that the work they do in scavenging, and in keeping down the vast quantities of bacteria that threaten to choke the passage-way of the intestine, quite outweighs any trifling inconvenience that their presence might possibly cause the host. The largest and healthiest-

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[1] Léger (1892) recorded three gregarines. I have already recorded, in a preliminary note ('Parasitology,' 1912), the following flagellates from Tipula: Trichomastix sp., Monocercomonas melolonthæ (Grassi), Polymastix melolonthæ (Grassi), Hexamitus intestinalis Duj., Embadomonas agilis Mackinnon, and E. alexeieffi Mackinnon. To these I would now add Rhizomastix gracilis Alexeieff and Tetratrichomastix parisii, n. sub-gen., n. sp. A paper dealing with the amœbæ is at present in preparation.

looking grubs were generally found to have the richest intestinal fauna. Probably such an association should rather be looked on as one of symbiosis. Certain of these protozoa have no doubt become closely adapted to their peculiar environment, and could flourish there only ; but others of them, such as the flagellates Polymastix and Monocercomonas, are found in other larval insect hosts besides Tipula ; while some are even less particular in their choice of host. The occurrence of these last is always sporadic and quite unpredictable. These are the " parasites facultatifs," which are of special value in helping us to fill in the gap between strict parasites and free-living forms.

To such a group of " facultative " parasites belongs the beautiful little monoflagellate, named by Alexeieff (1912) Rhizomastix gracilis. Alexeieff based his description on a single infection of an axolotl. I have recently found in Tipula a cercomonad which seems to me morphologically indistinguishable from Rhizomastix gracilis, and I see no good reason for " creating " a new species, even though the host be in the one case a vertebrate, and in the other an invertebrate. The infected leather-jackets were found in swampy ground near a stream—in much the sort of environment, that is to say, which would also be suitable for amphibians. I share with a few other protistologists the belief that animals totally unrelated, but frequenting the same feeding grounds, are liable to infection by the same " facultative " protozoan parasites. It would be well if the enthusiastic creators of " new species " would keep this possibility in mind.

The material at my disposal for the study of Rhizomastix was not much richer than that from Alexeieff's axolotls. Only two larvæ out of hundreds were found infected, and of these one so sparingly that only a dozen flagellates were found in the stained preparation. But in the other the organism was relatively abundant, and I found a large number of its cysts. I am thus able to add something to the little-known life-cycle of this cercomonad, and though the account is necessarily incomplete, I do not hesitate to publish it, believing as I do

in the extreme interest to protistologists of such erratic parasitic forms.

(a) Rhizomastix Gracilis Alexeieff.

The Flagellate Form.

A full description of the adult flagellate has been given by Alexeieff (1911). I quote the essential points. The form of the body is generally elongated, but may become globular; the posterior end is especially "metabolic." The single flagellum is about three times as long as the body; a small basal granule marks its emergence[1]; it is continued into the body by a sort of dark-staining filament, the rhizostyle, "qui atteint presque la longeur du corps et qui diminue brusquement de calibre en passant au voisinage du noyau, comme si une partie de la substance de la baquette s'arrêtait à la membrane nucléaire." The nucleus is very large, with voluminous karyosome, usually central; the peripheral chromatin is in the form of granules, connected with the karyosome by linin strands. The cytoplasm shows the clear alveolar structure characteristic of Cercomonas.

Alexeieff gives no measurements. The measurements I took showed the dimensions of the elongate flagellate individuals to be from $6\,\mu \times 3\,\mu$ to $11\,\mu \times 5\,\mu$. The diameter of the large round forms was about $6\,\mu$ to $8\,\mu$.

Figs. 1–7 (Pl. 31) show the appearance of the flagellate Rhizomastix gracilis as I found it in Tipula.

Encystment.

The oval bodies, "en bouteille, à goulot très court," which Alexeieff first described as the cysts of Rhizomastix (1911a), he referred later (1911b) to Chilomastix caulleryi.

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[1] This basal granule mentioned by Alexeieff appears rather as a thickening of the rhizostyle than as a "granule"; sometimes it is possible to see a minute granule at the inner termination of the rhizostyle within the body of the organism, see figs. 2, 4 and 6.

The course of encystment seems to be as follows. The organism loses its flagellum (Pl. 31, fig. 8), but the rhizostyle usually persists. The body becomes rounded off, and there appear in the cytoplasm, hitherto finely alveolar, two or three large vacuoles (Pl. 31, figs. 9 and 10). These vacuoles run together, and form one very large vacuole, which lies to one side of the now spherical cyst. By this time a definite cyst-wall, "à double contour," has formed. The diameter of the finished cyst is 5 to 6 μ. The nucleus lies between the cyst-wall and the vacuole (Pl. 31, fig. 11). At this stage it still retains the characteristic features of the adult flagellate nucleus, with a large central karyosome, and peripherally arranged chromatin granules, from which linin threads extend towards the centre, like the spokes of a wheel. The rhizostyle is still clearly visible as a dark-staining line, often equatorial in position. About this time it would appear that chromatin escapes into the cytoplasm from the nucleus ; the cyst contents stain darkly, especially just within the cyst-wall. This suggests that possibly the nuclear material plays some part in the formation of this envelope.[1]

The nucleus seems to swell, and its outlines become vague. The karyosome breaks up into a number of small round masses (Pl. 31, figs. 12 and 13). A spindle now makes its appearance, on which the karyosome granules occupy the position of the equatorial plate (Pl. 31, figs. 14 and 15). It is not clear what becomes of the peripheral chromatin. Possibly it unites with the central mass, but as the cytoplasm stains even more intensely and contains more dark granules than before, I think it possible that a good deal of the peripheral chromatin is absorbed.

Though the spindle is perfectly well defined, I have found no indication of centrioles at its poles. In one cyst (Pl. 31,

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[1] Cf. Alexeieff's observations (1913a) on the part played by chromidia in the cyst-formation of Chilomastix and other protozoa. Hartmann and Whitmore (1910) think that the extrusion of chromatin from the nucleus of Entamœba coli has something to do with the formation of the large central vacuole in the cyst.

fig. 16) there certainly appeared to be a group of granules at each end of the spindle, but this was probably chromatin that had travelled there from the equatorial plate, or else the residue of the peripheral chromatin.[1]

Gradually the spindle lengthens, and the chromatin from the middle separates towards the two poles, there forming dense, dark-staining, club-shaped masses. The spindle extends right across the cyst, curved against the bulge of the vacuole. It thins out and becomes vague, then disappears, leaving the two nuclei. These are at first exceedingly small and compact (Pl. 31, fig. 20), but they presently swell out, and take on the characters of the normal flagellate nucleus, with peripheral chromatin and a voluminous karyosome. As a rule the karyosome is not central, but lies pressed against the nuclear membrane(Pls. 31 and 32, figs. 22–25). The daughter-nuclei sometimes lie close together, sometimes at opposite sides of the cyst, which now is inclined to lose its regular circular outline (Pl. 32, fig. 26).

The rhizostyle has meanwhile divided into two. Sometimes this division takes place very early, even before that of the nucleus, sometimes later. This division does not seem to be a splitting of the whole structure; division of the basal granule is followed by the gradual growth of a second rhizo-style from one of the daughter-granules (Pl. 31, figs. 13, 23 and 24).

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[1] If the recent "systematisation" of primitive mitosis given by Alexeieff (1913) is to be followed, then the mode of nuclear division in the cysts of Rhizomastix gracilis comes near to that named mesomitosis. "Centrioles aux deux pôles du noyau en division; la plaque équatoriale, formée par le caryosome, massive au début, se morcelle en un certain nombre de chromosomes qui . . . se repartissent aux deux pôles où ils se confondent avec les centrioles." But I have been unable to demonstrate centrioles in Rhizomastix, and in this respect Alexeieff's rheomitosis, in which the centrioles are early hidden by accumulating chromatin granules, seems to meet the case better—"à la place de centrioles on observe des corps chroma-tiques de plus en plus volumineux et finalement on se trouve en présence d'une apparence de promitose avec ses corps polaires."

This stage, with two large nuclei, a large vacuole, and clear alveolar cytoplasm, is much the most abundant. I have looked in vain in my small material for the fission of the cytoplasm which I believe follows, and for the escape of the two flagellates. Probably the cysts are passed out from the gut of the host in this binucleate state.

In the second of my preparations, where there were no cysts seen, ten of the twelve small flagellates were found with the karyosome eccentric, and possibly they had recently emerged from cysts (Pl. 31, figs. 4–7; Pl. 32, figs. 27–29). In these forms it was often impossible to trace the rhizostyle past the nucleus, into the anterior border of which it appeared to be inserted. (Cf. the flagellar apparatus in Oicomonas [see Hartmann and Chagas, 1910], to which this arrangement is very similar.) In one small individual (Pl. 32, fig. 29) there were three nuclei, but I do not know what this indicates.

In the cysts I have found no trace of fusion of the nuclei, nor any indication that there is a sexual process there. They seem to be simply multiplication cysts.

Systematic Position.

As Alexeieff suggests (1911A), the affinities of Rhizomastix are probably on one side with free-living forms like Oicomonas and Cercomonas,[1] and on the other with parasites like Herpetomonas and its allies. There is a striking resemblance between Oicomonas, as figured by Hartmann and Chagas, and certain flagellate stages of

[1] I am unable to appreciate Alexeieff's reasons (1911B) for removing Oicomonas from among the Cercomonadines. He speaks of its mode of encystment as resembling that of Monas. At present our knowledge of the encystment of Oicomonas and its allies is so scanty as to afford little reliable indication of their affinities, but I do agree that in the comparative study of such processes lies our surest hope of ultimately bringing something like order out of the present chaos of flagellate classification. "Die Kenntniss der Entwicklung ist das erste Postulat der Protozoenforschung" (Schaudinn).

Rhizomastix. The rhizostyle of Rhizomastix is very possibly homologous with the much-debated fibril " Doppel-faden," that Prowazek was the first to demonstrate in Herpetomonas muscæ-domesticæ.

My account of the encystment does not add much to help in determining the systematic position further. Cysts of Cercomonas have been described by Hartmann and Chagas (1910), Wenyon (1910), and Alexeieff (1911), but those seem in every case to be mere " Schützcysten," in which no division phenomena take place. Doflein (1910) and Ogawa (1913) have described and figured certain globular forms which occurred in their cultures of Trypanosoma rotatorium. I have been struck by the resemblance of these to the cysts of Rhizomastix. One of Doflein's figures in particular shows a large vacuole filling out the bulk of the sphere, and crushing aside the cytoplasm, which contains two nuclei. There are kinetonuclei present, of course, and there is no cyst-wall; in other respects the picture is not unlike what I have shown in Pl. 32, fig. 26. Ogawa describes these as " Ruhe oder Dauerformen."

It is very difficult to say how far a similar mode of nuclear division indicates affinity between protozoa. The division of Rhizomastix within the cyst does not bear much resem-blance to that of Cercomonas flagellates as described by Hartmann and Chagas and others. On the other hand, the division of Trypanosoma lewisi is a mesomitosis according to certain authors.

If importance is to be attached to such points as these, then the study of Rhizomastix gracilis tends to strengthen the view that the Trypanosomidæ Doflein (= Herpetomon-adidæ Alexeieff) are derived from Cercomonadine ancestors.

(b) TETRATRICHOMASTIX PARISH, N.SUB-GEN., N. SP.

General Considerations.

Though the trichomonad flagellates have received much attention from a number of workers during the last few years,

there is still considerable difference of opinion as to the systematic value of the flagellar apparatus. On the one hand, Doflein (1911) considers that the genera Tricho-monas and Trichomastix are just varieties of the same form, of which the recurrent flagellum is attached to the body-wall in the first case, while it remains free in the second. On the other hand, there are those, like Parisi (1910), who think that the number and mode of attachment of the flagella are points of real value to the classifier of these organisms. Parisi, accordingly, has split up the genus Trichomonas Donné into three sub-genera :

(1) Trichomonas sensu stricto, with three anterior flagella, and an undulating membrane.

(2) Tetratrichomonas, with four anterior flagella, and an undulating membrane.

(3) Trichomastix, with three anterior flagella, and one recurrent flagellum, which does not adhere to the body to form an undulating membrane.

The flagellate which I am about to describe from Tipula, while undoubtedly a trichomonad, falls into none of these three sub-genera. It is provided with five flagella, of which four are directed forwards, and the fifth is a " Schleppgeissel," as in Trichomastix. This form, then, bears exactly the same relations to Trichomastix that Tetratrichomonas has to Trichomonas.[1] I propose to place it in a fourth sub-genus, Tetratrichomastix. This seems the simplest method of proceeding, so long as we abide by the present classification of the trichomonads.

To subdivide the genus is certainly a convenience, and the characters on which the subdivision is based seem clear enough. What is less satisfactory are the " species " within these sub-genera. I have already expressed my scepticism with regard to what may be called " host-species " in para-sitic protozoa. Some authors consider that flagellates should

[1] A true Trichomastix with three anteriorly directed flagella, indistinguishable from Trichomastix trichopterorum, Mackinnon, also occurs in Tipula (see Mackinnon, 1912).

be classified upon morphological grounds alone. Alexeieff (1911) gives five characters, which, taken together, should form, he considers, a reliable basis for classifying the species of Trichomonas. These deal with such points as the dimensions of the axostyle, the structure of the nucleus, the distribution of the extra-nuclear siderophilous granules, etc., and they must be studied, he says, on the adult flagellate. Now these are characters that are subject to some fluctuation, particularly if the organism be a parasite of more than one kind of host; moreover, it is by no means always easy to decide which is the typical adult form. In the case of Trichomastix, two of the said characters, i.e. the para-basal body and the condition of the undulating membrane, are necessarily absent, and this renders the species determin-ation on morphological grounds increasingly uncertain. What makes the difficulty still greater is that slightly stronger differentiation of iron-hæmatoxylin preparations produces such difference in the degree to which the "species" characters are exhibited.

In the present case two forms of Tetratrichomastix occur (vide infra), but I hesitate to dub them species. As these two forms of the flagellate never occur side by side in the same preparation, I am strongly of the opinion that the degree of intensity of the staining must be taken into account. For the present I propose to consider them as forms of one flagellate—Tetratrichomastix parisii, n. sub-gen., n. sp.

Diagnosis.

Tetratrichomastix n. sub-gen. Flagellate with axostyle and five free flagella, four anteriorly, and one posteriorly directed.

T. parisii n. sp. Axostyle slender and rather poorly developed. Nucleus oval or round, (1) compact, rich in chromatin blocks (form A, Pl. 32, figs. 30 and 31), or (2) feebly siderophilous, with a few scattered chromatin granules and a well-defined nuclear membrane (form B, Pl. 32, figs. 32 and

33). In form A the nucleus is often surrounded by a halo of small, siderophilous, drop-shaped bodies (ingested bacteria?). In form B, the cytoplasm contains several large vacuoles, and stains relatively intensely. No cytostome visible. Dimensions $8\,\mu$ by $4\,\mu$ to $12\,\mu$ by $7\,\mu$.

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EXPLANATION OF PLATES 31 AND 32,

Illustrating Miss D. L. Mackinnon's paper on (a) "The Encystment of Rhizomastix gracilis Alexeieff; (b) Tetratrichomastix parisii n.sub-gen., n.sp."

[Figures drawn to scale (× 4000 ca.) under Zeiss comp. oc. 12 and 2 mm. apochromat. Figs. 7, 8, 27, 28 and 29 are on a slightly smaller scale than the rest. The stain employed was Heidenhain's iron-hæmatoxylin, after fixation with sublimate alcohol.]

Figs 1–29. Rhizomastix gracilis.

Fig. 1.—Large pear-shaped flagellate individual, showing single long flagellum (*Fl.*), " rhizostyle " (*Rh.*), basal granule of Alexeieff (*B. G.*), and large vesicular nucleus (*Nu.*).

Fig. 2.—Flagellate of more spherical form. The granule at the inner end of the rhizostyle is well shown here (*G.*).

Fig. 3.—Flagellate of which the nucleus has an eccentric karyosome.

Figs. 4–7.—Various forms assumed by Rhizomastix gracilis. Note the clear zone round the nucleus, probably an artefact and due to shrinkage. In figs. 4, 5 and 6 the cytoplasm shows numerous inclusions, probably bacteria. In figs. 5 and 7 the rhizostyle has much the same relations to the nucleus as in Oicomonas.

Fig. 8.—Rounded-off individual without flagellum. Resting stage?

Fig. 9.—Preparation for encystment. The flagellate rounds off, and its cytoplasm becomes more coarsely vacuolated.

Fig. 10.—A rather later stage. The flagellum has disappeared : the vacuoles become larger and fewer in number.

Fig. 11.—The cyst formed. Note the large vacuole (*Va.*) and the persistent rhizostyle (*Rh.*).

Fig. 12.—The nucleus swells, and there is an escape of chromatin into the cytoplasm.

Fig. 13.—The nuclear outlines become vague; the karyosome fragments; the rhizostyle has doubled.

Figs. 14–16.—Formation of the spindle. In fig. 16 a certain amount of chromatin has already collected at the poles.

Figs. 17–20.—The chromatin collects at the poles. The spindle thins out more and more.

Fig. 21.—The spindle has disappeared. Two compact nuclei are left.

Figs. 22–26.—Various aspects of the cyst with two nuclei. In fig. 22 the large vacuole has disappeared. In fig. 26 one of the nuclei has taken on the vesicular form, and the cyst has elongated.

Figs. 27–29.—Small flagellates with eccentric nucleus. Possibly these have recently emerged from cysts. Fig. 29 shows an individual with three nuclei.

Figs. 30–33. Tetratrichomastix parisii.

Fig. 30.—Form A. Note fully developed axostyle and dark-staining nucleus with large chromatin masses.

Fig. 31.—Form A. Note halo (*H*.) of drop-shaped bodies surrounding nucleus.

Fig. 32.—Form B. Note relatively large, faintly staining nucleus, poor in chromatin.

Fig. 33.—Form B. Flagellate without axostyle.

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MEMOIRS:

WITH TITLE, CONTENTS, AND INDEX TO VOL. 59.

The Development of Echinocardium cordatum.

Part I.—The External Features of the Development.

By

E. W. MacBride, F.R.S., V.P.Z.S.,
Professor of Zoology in the Imperial College of Science and
Technology.

With Plates 33 and 34.

ANYONE reading through the comprehensive summaries of our knowledge of echinoderm larvæ given by Mortensen (1898, 1901) must be struck on the one hand by the large number of varieties of echinoderm larvæ described therein, and on the other hand, by the paucity of cases in which the parentage of the larva had been accurately determined.

In the summer of 1911, when working at the laboratory of the West of Scotland Marine Biological Association, situated at Millport on the Isle of Cumbrae on the Firth of Clyde, I succeeded in artificially fertilising the eggs of Echinocardium cordatum, and in rearing the resulting larvæ through the whole period of their free-swimming life until they metamorphosed into young urchins. Last summer, (1913) I returned to Millport and repeated my experiments, and again succeeded in rearing the larvæ of this species through their entire development until the completion of metamorphosis. Drawings of the larvæ at every stage of their development were made from living specimens, and these drawings, slightly corrected by comparison with larvæ preserved and mounted whole, have been used to illustrate this paper.

I had originally intended to delay publication until I should have had leisure to work out the internal features of develop-

ment by means of sections, but the necessary leisure for this seems still to be a long way off, and it seems worth while in the meantime to place on record the external features of the development of a Spatangoid.

Our knowledge of the complete life-history of echinoid larvæ is still extremely scanty; indeed, the only species in which the external features of the development have been completely described are the Clypeastroid, Echinocyamus pusillus, described by Théel (1892), the endocyclic urchins Echinus esculentus and E. miliaris described by myself (1903), and quite recently Strongylocentrotus lividus described by von Übich (1913).

The larva of Echinocardium cordatum was first seen by Johannes Müller (1846). He described the later phases of larval life immediately preceding metamorphosis: he was able to identify the larva as the larva of some echinoid but which echinoid he was quite unable to say. His figures, however, leave no doubt in my mind that he was dealing with the larva of Echinocardium cordatum. The early larva of Spatangus purpureus was described by Krohn (1853), who reared it from artificially fertilised eggs, and his work has been repeated and confirmed by Mortensen (1913), who kept the larvæ alive for three weeks.

The younger stages in the larval development of Echinocardium cordatum were described by Vernon (1898) and more recently by myself (1912). The fully developed larva was described and figured by Mortensen (1901) but the identification of his specimens as the larvæ of Echinocardium cordatum rests on a mere guess because the ground of the identification was the occurrence of the larva in question in the waters of the Lymfiord in Denmark on the sandy bottom of which Echinocardium cordatum was the only Spatangoid which was found.

I have to express my thanks to the Director of the Millport Station, Mr. Richard Elmhirst, for his kindness to me during my stay in Millport and for the assistance which he rendered me in carrying out my experiments.

I am also especially indebted to Dr. Gemmill, Lecturer in Embryology in the University of Glasgow and Vice-President of the West of Scotland Marine Biological Association, for the pure cultures of the diatom Nitschia with which he supplied me both in 1911 and in 1913. To these cultures I ascribe my success in rearing the larvæ through the whole of their development until the completion of metamorphosis.

The methods adopted were exceedingly simple. The laboratory at Millport is situated close to the fairway of the Firth of Clyde, and the water which streams up the firth with every incoming tide is quite uncontaminated and perfectly suitable for rearing the larvæ; the only precaution which it is necessary to observe is to procure the water a few yards from shore and to convey it to the laboratory in an earthenware or glass vessel, since sea-water rapidly contracts contamination from metallic vessels, and for this reason the sea-water which is pumped into the tanks at Millport is unsuitable, since it passes through metallic pipes before reaching the laboratory.

The entire development up to the completion of metamorphosis occupies a period of about four weeks, and is, therefore, very rapid as compared with the development of Echinus esculentus, for in this latter species at least six weeks must elapse before the metamorphosis is completed, and under laboratory conditions a period of two months and more is not infrequently required before the imago condition is attained.

A brief résumé of the times (reckoned from the moment of fertilisation of the egg) required for the attainment of well-marked stages in the developmental history of Echinocardium cordatum is given below :

Free-swimming blastula . . .	10 hours
Formation of primary mesenchyme and incipient gastrulation	15 ,,
Gastrulation completed	30 ,,
Formation of cœlom and first trace of stomodæum, the post-oral (anal) arms of the larva formed	2 days

Formation of antero-lateral arms and of
    aboral process . . . . . 3 days
First trace of postero-dorsal arms . . 4 „
Postero-dorsal arms fully developed ; first
    trace of " Echinus rudiment " and of
    præ-oral arms . . . . . 9 „
Hydrocoele with incipient lobes; first
    trace of postero-lateral arms . 10–12 „
Formation of antero-dorsal arms ; tube-
    feet protruding with amniotic cavity 14–16 „
Formation of adult spines; absorption of
    aboral process . . . . 18–22 „
Metamorphosis complete . . . 24–30 „

The egg is not a sphere but an ellipsoid, and its shape corresponds to that of the blastula which is developed from it (fig. 1). The egg of Echinus is spherical and gives rise to a spherical blastula; it follows that the shape of the blastula, since it is conditioned by the shape of the egg, is always a maternal character. At the aboral pole of the blastula of Echinocardium there is a patch of thickened epithelium consisting of long, narrow, filamentous cells bearing long cilia (*ap.*, fig. 1) ; this patch, like the apical plate of the annelid larva, must be regarded as a sensory organ, the purpose of which is to direct the tiny larva in its course through the water. No such organ can be detected in the blastula of Echinus which progresses by a rolling motion, but in the gastrula of Echinus, in which the rolling motion gives place to motion along a definite line, a very similar organ can be detected.

As in other Echinoid larvæ mesenchyme cells (*p. mes.*, fig. 1) are given off from the pole of the blastula opposite the one at which the sensory organ is situated, before any sign of the invagination which is to form the archenteron can be detected. The main purpose of this mesenchyme—which is termed the primary mesenchyme, is to form the basis in which the rods of the larval skeleton are secreted. By the close of the second day not only is the archenteron completely formed, but the

first rudiments of the larval skeleton have appeared (*calc.*, fig. 2). They are in the form of two calcareous " stars " situated to the right and left of the middle line. In each " star " one arm grows outwards and impinges on the ectoderm and so causes the formation of one of the first pair of arms of the larva. In Echinocardium this outwardly directed rod is accompanied by another rod parallel to it, which arises as a branch from another arm of the star. These parallel rods are connected at intervals by cross-bars, and are conveniently regarded as one compound latticed rod which receives the name of post-oral rod (*p. o. r.*, fig. 3), and which forms the skeleton of the post-oral arm. The post-oral arms which are thus the first of the larval arms to be formed are often termed anal arms by German writers on Echinoid development (*p. o. a.*, fig. 3). From the apex of the archenteron mesenchyme cells continue to be given off. These cells, which are termed secondary mesenchyme (*s. mes.*, fig. 2), form the wandering cells which traverse the blastocele or primary body-cavity of the larva and also form the delicate and sparse connective tissue which is found in this cavity later. We have seen that the post-oral rods arise, each from an outwardly-directed branch of one of the calcareous stars. The other branches of the calcareous stars grow in length, and one which extends downwards towards the lower pole of the larva is termed the body-rod; another which extends horizontally inwards towards the middle line along the posterior surface of the larva is termed the horizontal rod. A third branch of each star which extends upwards towards the upper pole of the larva is termed the antero-lateral rod. At the lower pole of the larva there is to be seen a mass of mesenchyme cells wedged in between the lower ends of the two body-rods. In this mass there can be detected a minute calcareous star (*ab. calc.*, fig. 4) which is the rudiment of the skeleton of the aboral spike.

In the meantime a bilobed outgrowth appears at the apex of the archenteron; this is the rudiment of the cœlom. It soon becomes cut off and divided into right and left sacs (*cœ.*, fig. 4),

whilst the rest of the archenteron which forms the GUT, becomes marked out by constrictions into œsophagus, stomach and intestine.   Between the rudiments of the post-oral arms and the part of the body into which the nascent antero-lateral rods project there appears a concavity.   This concavity causes the part of the body supported by the antero-lateral rods to stand out prominently and we shall call it the oral lobe (o. l., figs. 3, 4, and 5).   The cilia which clothed the whole surface of the gastrula become now confined to a thickened band of epithelium which runs along the edge of the oral lobe and over the tips of the post-oral arms.   This is the longitudinal ciliated band, the order of locomotion in the larva (l. cil., fig. 3).

On the inner aspect of the oral lobe the stomodæum (stom., fig. 4) appears as a shallow depression; this soon becomes connected with the endodermal œsophagus and the alimentary canal is thus completed ; the blastopore of course persists as the anus.   All the changes are completed by the end of the second day.

Along the sides of the compound œsophagus a band of thickened epithelium makes its appearance.   This is the rudiment of the adoral ciliated band (ad. cil., fig. 6). During the third day the new centre of calcification mentioned above develops rapidly.   From one of its arms there is developed a latticed rod consisting of three parallel rods bound together by cross bars, which projects backwards and pushes out a corresponding club-shaped protrusion of the body-wall which is the well-known aboral spike of the spatangoid larva.   The latticed rod is known as the aboral rod (ab. r., fig. 5).   The antero-lateral rods also develop rapidly and push out a pair of protrusions of the ciliated band which are the rudiments of the antero-lateral arms (a. la., fig. 5).   From each antero-lateral rod there is given off a posterior branch which runs back towards the lower pole of the larva parallel to the body rod.   This branch is termed the recurrent rod (r. r., figs. 4 and 5).   In order to support the heavy aboral rod connections are formed between the recurrent and body

rods on each side and the lateral branches of the aboral rod (fig. 5), but the lower extremities of the body rods rest against the sides of the aboral rod without fusing with it (figs. 8, 9).

The little four-armed larva can now feed, and if placed under suitable conditions rapidly grows in size. Already by the end of the fourth day two additional calcareous centres appear, situated one on each side of the larva in the concavity between oral lobe and post-oral arms. Each of these centres has three rays—one directed outwards, one upwards and one downwards. From the outwardly directed ray there is developed a latticed rod consisting of two parallel bars connected by cross-bars. This rod is the postero-dorsal rod; and as it grows it pushes out a corresponding protrusion of the ciliated band which is the postero-dorsal arm (*p. d. a.*, fig. 6). By the eighth or ninth day this arm has grown to two thirds the length of the corresponding post-oral arm and another calcareous centre has appeared. This latest centre is a median unpaired crescent situated on the dorsal side of the œsophagus at the base of the oral lobe. It is termed the dorsal arch (*d. a.*, fig. 6) and in succeeding days it furnished by the growth of its two ends, two rods termed the præ-oral rods which form the skeleton of the two præ-oral arms. These last-named arms are situated in front of the mouth just beneath the antero-lateral arms and slightly nearer the middle line (*pr. o. a.*, fig. 6), and it is a noteworthy circumstance that whereas the other arms only appear when the corresponding rods of the larval skeleton impinge on the ectoderm, the rudiments of the præ-oral arms appear before the branches of the dorsal arch reach them. The formation of the arm is certainly not due to a mechanical stretching of the ectoderm by the growing rod beneath it, for in unhealthy larvæ the ectoderm can become contracted and the rod projects from the apex of the arm as a bare spine; we must rather picture the growth of the ectoderm which forms the arm as the response to a chemical stimulus emitted by the calcareous rod, and in the case of the præ-oral arms we must

admit that their first beginnings are entirely independent of the origin of the skeleton.

Important internal changes have meanwhile taken place. The cœlomic sac on each side has become divided into anterior and posterior portions, the anterior situated at the side of the larval œsophagus, the posterior at the side of the larval stomach. On the left side the anterior division has given origin to a posterior thick-walled outgrowth; this is the hydrocoele (*hy.*, fig. 6), the rudiment of the adult water-vascular system. This lies just beneath the ectoderm on the left side in the concavity between postero-dorsal and post-oral arms. An invagination of the ectoderm, which will form the amniotic cavity, appears just over the spot where the hydrocele is situated and becomes closely applied to it. The two adpressed structures form a compound rudiment which I have termed the Echinus-rudiment (1903), and by the growth of this rudiment the oral disc of the adult is formed in the manner described by me (1903). The larval œsophagus is surrounded by a band of circular muscles by the aid of which the peristaltic movements involved in swallowing are carried on. These muscles (*musc. circ.*, Pl. 34, fig. 15) are developed from the walls of the anterior divisions of the cœlom. The larva has now attained a stage in development which in exceptional cases may be attained by a larva of Echinus esculentus in the same time, but which more frequently requires sixteen or seventeen days for its accomplishment in the latter species.

From this point the development of Echinocardium and Echinus begin to diverge more and more. For whereas in the larvæ of the latter genus horizontal crescentic bands bearing very strong cilia begin to be differentiated from the longitudinal ciliated band, and the aboral ends of all the rods of the larval skeleton begin to be absorbed, in larvæ of Echinocardium neither of these changes take place, but on the contrary by the ninth or tenth day a new pair of larval arms, unrepresented in the Echinus larva, begin to make their appearance. These are the postero-lateral

arms (*p.l.a.*, fig. 8), which arise from near the base of the
aboral spike and which extend horizontally outwards.   Each
is supported by a postero-lateral rod (*p.l.r.*, fig. 7), which
is in reality only one of the lateral branches of the aboral
calcareous star which has grown outwards.   At the apex of the
aboral spike there is a crest of ciliated epithelium (*ab. cil.*, fig.
8) which is entirely independent of the principal longitudinal
ciliated band.   By this time the hydrocoele has become marked
out into incipient lobes which are the rudiments of the
radial water-vascular canals and of the primary tube-feet of
the adult (*hy. l.*, fig. 8), and the amniotic invagination has
become converted into a closed amniotic cavity (*am.*, fig. 8).

By the end of the second week a sixth and last pair of
arms have made their appearance.   These are the antero-
dorsal arms; they spring from the dorsal surface of the
oral lobe near its base and each is supported by an antero-
dorsal rod which arises as a branch from the corresponding
præ-oral rod.   The larva has now attained its full complement
of arms, viz. twelve, or if we count the aboral spike as an arm,
thirteen, and for this reason Johannes Müller termed it the
"Pluteus with thirteen arms."   To judge from Mortensen's
(1913) figure it differs from the larva of Spatangus of corre-
sponding age in the fact that in the latter larva the aboral spike
is as long as the rest of the body including the præ-oral arms,
whereas in the larva of Echinocardium, as fig. 9 shows, the
aboral spike is only one fourth as long as body including
præ-oral arms.   The living larva at this stage is a marvel-
lously beautiful object.   All the thirteen arms are dotted
over with patches of bright red pigment which is especially
massed near their tips, and interspersed with the patches of
this pigment are patches of a light yellowish-green pigment,
and the two pigments combine to give the larva a wonderfully
gay appearance.

The fully developed larva of Echinocardium has thus
four larval arms not possessed by the larva of Echinus and
in addition the aboral spike.   Nevertheless it only possesses
one more centre of calcification than is present in the larva of

Echinus and that is the centre for the aboral spike. This furnishes not only the aboral rod but also the postero-lateral rods, whilst the dorsal arch, as we have seen, supplies the skeleton for the antero-dorsal as well as for the præoral arms.

During the third week the aboral spike begins to be absorbed. This absorption takes place at the tip; the ciliated crest is lost and the aboral rod becomes shorter. The absorption seems to be carried out by the agency of the cells carrying the red pigment, for these are seen thickly massed about the free end of the spike. The other arms grow longer so that the tips of the præ-oral, antero-lateral, postero-dorsal and post-oral arms extend to about the same distance from the body taking the web of skin connecting the post-oral arms as the base-line. Two large vacuities (vac., fig. 11) appear in this web one on each side. The other arms, viz. the antero-dorsal and postero-lateral, grow much longer but do not attain the length of the rest. The rods supporting all these arms become much attenuated so that they actually become flexible. The Echinus-rudiment grows in size and the lobes of the hydrocœle begin to appear as tentacles projecting into the amniotic cavity (ten., fig. 10). From the floor of the amniotic cavity there grow up other protrusions; these are the rudiments of the spines of the adult (ad. sp.). Outside the amniotic cavity there begin to appear alternating circles of calcareous stars. These are the rudiments of adult plates belonging to the aboral region of the adult (ab. p., figs. 12 and 13).

On the dorsal surface of the oral lobe an epithelial thickening can now be seen. This is almost certainly homologous with the organ which I have described (1903) as the larval brain in the larva of Echinus esculentus. The corresponding organ in Echinocardium cordatum shows two outgrowths which pass towards the halves of the longitudinal ciliated band—a fact which indicates that the probable function of the organ is to co-ordinate the activities of the two halves of the ciliated band (l. b., fig. 12)

Towards the end of the third week the Echinus-rudiment

has grown in size so much that it not only takes up the entire left side of the larva, but extends round on to the dorsal surface and also on to the ventral surface of the larva, displacing the anus (a., fig. 15) to the right side. It becomes thickly studded with conical adult spines, which fit so closely together that the whole presents the aspect of a tesselated pavement (fig. 11). In two points the larva differs conspicuously from the larva of Echinus esculentus at the corresponding stage. (1) The larva of Echinus develops a series of plates on its right side which bear small four-sided clavate spines and three pedicellariæ, whereas the larva of Echinocardium shows no trace of these extra-amniotic spines and pedicellariæ. (2) The echinus-rudiment in the larva of Echinus grows in size but is confined to the left side and it develops only four adult spines in each interradius, whereas in the larva of Echinocardium the Echinus-rudiment develops many spines and extends far over both on to the dorsal and ventral surfaces of the larva (figs. 14 and 15).

At some time during the fourth week the critical epoch in the development of Echinocardium cordatum is reached and the metamorphosis of the larva into the imago takes place with startling rapidity. I have often watched a larva with all its processes except the aboral spike fully developed, change into an urchin inside half an hour. The roof of the amniotic cavity, which we may term the amniotic veil, becomes torn, and the tentacles and spines of the Echinus-rudiment emerge. The epithelial skin of the larval arms flows back into the body leaving the supporting rods to project as bare spines. I use the word " flow " advisedly for this is the appearance which is presented : the epithelial covering often becomes massed into drops, and it is at once evident that the separation of its component cells one from another is a mere temporary and superficial phenomenon, and that the epithelium as a whole behaves like a film of fluid. The projecting spines soon become broken off and the little urchin becomes launched on its career. The adult mouth appears to be open from the beginning, whereas in Echinus it is covered with an epi-

neural veil which is only perforated some days later. The outline of the urchin is ellipsoidal, not circular as in the imago of Echinus, and whereas in Echinus the oral surface is flat and the five primary tube-feet are the principal organs of locomotion, in Echinocardium on the contrary the young urchin moves about by the aid of its spines and the oral surface is rounded. A curious consequence of this difference in mode of locomotion is seen in the condition of the water-vascular system in the late larvæ and young imagines of the two genera. In Echinus this system is gorged with fluid for the tentacles are very extensible. In Echinocardium the lumina of the water-vascular ring and tentacles are very narrow since the tentacles are only extensible to a slight extent.

There is another great difference between the young imagines in the case of the two genera. In Echinocardium the five primitive tube-feet, which, it must be remembered, represent the apical terminations of the radial canals, arise from near the mouth, and all the spines are situated further from the mouth, and apparently must belong to the apical region of the fully developed adult. In Echinus, on the contrary, the tube-feet arise from the edge of the oral surface of the young imago, and the pointed spines are situated as near or nearer to the mouth, and hence belong to the region of the corona in the fully developed adult. Only the square-topped spines which arise outside the amniotic area belong to the apical region of the fully developed adult. Hence the conclusion inevitably follows that the floor of the amniotic cavity does not represent the same region in the two genera, and the speculation is awakened that perhaps in the far-off common ancestor of Echinus and Echinocardium no amnion was developed at all, but the spines and tube-feet of the adult were developed in an exposed condition on the side of the larva, as is the case in Asteroidea.

Most of the spines of the imago of Echinocardium are conical, but there occur at one end of the ellipsoidal imago a short crescent of shorter flattened club-shape spines (*cl. sp.*,

fig. 16). These I regard as the first prophecy of the flattened spines of the adult. Surrounding the mouth and alternating with the tube-feet are five short inwardly directed spines. These I regard as the homologues of the teeth of regular Echinoids (*dent.*, figs. 16 and 17). Whilst reserving an account of the internal anatomy of the larva for another paper, I may say here that sections show that these "teeth," unlike the other spines, have roots which project inwards into dental pockets, as do the teeth of regular Echinoids. All the spines have thick collars round their bases. These collars consist of nervous epithelium, underneath which are the muscles connecting the spines to the plates of the skeleton.

For a short time there persists a trace of the oral lobe of the larva with short stumps representing the remnants of the antero-lateral and præ-oral arms and a shallow pit representing the last trace of the larval stomodæum, which has become disconnected from the œsophagus (*ol.*, fig. 16). Soon, however, this also disappears. Some of the young urchins lived for a week and grew in size. In one of these (shown in figs. 17 and 18) the five first sphæridia have appeared (*sph.*, fig. 17). These are situated outside the teeth in an adradial position, each sphæridium lying beside a tube-foot. These sphæridia, therefore, cannot correspond to the five inter-radial sphæridia of the adult; the post larval history of Echinocardium is quite unknown and a study of it would throw light on many important problems.

In reviewing the life-history which has just been described it is evident that we are dealing with a type of development much more modified than that of Echinus, and one in which the features of the adult appear at a still earlier stage than is the case with that genus.

IMPERIAL COLLEGE OF SCIENCE;
    November 27th, 1913.

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EXPLANATION OF PLATES 33 AND 34,

Illustrating Prof. E. W. MacBride's paper on " The Development of Echinocardium cordatum.

LIST OF ABBREVIATIONS EMPLOYED.

a. Larval anus. *ab. calc.* Calcareous centre for formation of aboral rod. *ab. cil.* Aboral crest of cilia. *ab. mes.* Aboral mass of mesenchyme. *ab. pl.* Rudiment of plate belonging to the aboral surface of the adult. *ab. r.* Aboral rod. *a. d. a.* Antero-dorsal arm. *ad. cil.* Adoral band of cilia. *ad. sp.* Adult spines. *a. l. a.* Antero-lateral arm. *a. l. r.* Antero-lateral rod. *am.* Amniotic cavity. *a. m.* Adult mouth. *a.p.* Apical plate of the early larva. *b. r.* Body-rod. *calc.*

Calcareous centre for the formation of the larval skeleton. *cl. sp.* Clavate spine of the adult. *cœ.* Cœlom sac. *d. a.* Dorsal arch. *dent.* Spine possibly homologous with the tooth of a regular Echinoid. *h. r.* Horizontal rod. *hy.* Hydrocœle. *hy. l.* Lobe of hydrocœle. *int.* Intestine. *l. a.* Vestiges of larval arms. *l. a. c.* Left anterior cœlom. *l. b.* Larval brain. *l. p. c.* Left posterior cœlom. *œs.* Œsophagus. *o. l.* Oral lobe of the larva. *p. d. a.* Postero-dorsal arm. *p. d. r.* Postero-dorsal rod. *p. l. a.* Postero-lateral arm. *p. l. r.* Postero-lateral rod. *p. mes.* Primitive mesenchyme. *p. o. a.* Post-oral arm. *p. o. r.* Post-oral rod. *pr. o. a.* Præ-oral arm. *r. a. c.* Right anterior cœlom. *r. p. c.* Right posterior cœlom. *r. r.* Recurrent rod. *s. mes.* Secondary mesenchyme. *sph.* Sphæridium. *st.* Stomach. *stom.* Stomodæum. *ten.* Primary tentacle of the water-vascular system. *vac.* Vacuity in the web connecting the two post-oral arms.

Fig. 1.—Blastula 15 hours old. × 200. *ap.* Apical plate carrying specially long cilia. *p. mes.* Primitive mesenchyme.

Fig. 2.—Gastrula about 32 hours old. × 200. *calc.* Centre of calcification for the larval skeleton. *s. mes.* Secondary mesenchyme.

Fig. 3.—Larva 2 days old viewed from the ventral surface. × 200. *a.* Larval arms. *ab. mes.* Mass of mesenchyme in which the skeleton of the aboral spike is formed. *b. r.* Body-rod of skeleton. *cœ.* Unpaired rudiment of the cœlom. *h. r.* Horizontal rod of skeleton. *p. o. a.* Rudimentary post-oral arm. *p. o. r.* Post-oral rod.

Fig. 4.—Larva of same age as last, but more advanced in development, viewed from the dorsal surface. × 125. *ab. calc.* Calcareous centre for the formation of the aboral rod. *a. l. r.* Antero-lateral rod. *cœ.* Cœlomic sac. *r. r.* Recurrent rod. *stom.* Stomodæum.

Fig. 5.—Larva 3 days old viewed from the dorsal surface. × 125. *ab. r.* Aboral rod. *a. la.* Antero-lateral arm. *œs.* Œsophagus. *st.* Stomach.

Fig. 6.—Larva 8 days old viewed from the dorsal surface. × 125. *ad. cil.* Adoral ciliated band. *d. a.* Dorsal arch. *hy.* Hydrocœle. *p. d. a.* Postero-dorsal arm. *p. d. r.* Postero-dorsal rod. *pr. o. a.* Præoral arm.

Fig. 7.—Larva 9 days old viewed from the ventral surface. × 125. *p. l. r.* Postero-lateral rod.

Fig. 8.—Larva 9 days old a little more advanced in development than that represented in fig. 7 viewed from the dorsal surface. × 125. *ab. cil.* Aboral crest of cilia. *am.* Amniotic cavity. *hy. l.* Lobes of the hydrocœle. *p. l. a.* Postero-lateral arm.

Fig. 9.—Larva 16 days old viewed from the dorsal surface. × 100. *a. d. a.* Antero-dorsal arm.

Fig. 10.—Larva 18 days old viewed from the dorsal surface. × 75. *ten.* Primary tentacles of the water-vascular system.

Fig. 11.—Larva 22 days old just about to metamorphose viewed from the ventral surface. × 75. *ad. sp.* Spines of adult. *vac.* Vacuity in the web of skin connecting the post-oral arms.

Fig. 12.—Central portion of a larva 20 days viewed from the dorsal surface. × 125. *ab. p.* Rudiments of plates belonging to the aboral surface of the adult. *l. a. c.* Left anterior cœlom. *l. b.* Larval brain. *l. p. c.* Left posterior cœlom. *r. a. c.* Right anterior cœlom. *r. p. c.* Right posterior cœlom.

Fig. 13.—Central portion of a larva 20 days old viewed from the ventral surface. × 125. *int.* Larval intestine. *vac.* Vacuity in the web of skin connecting the post-oral arms.

Fig. 14.—Dorsal view ⎱ of the central portion of a larva of 23 days
Fig. 15.—Ventral view ⎰ about to metamorphose. × 125. *ad. sp.* Spines of the adult.

Fig. 16.—Oral view of a just metamorphosed imago. × 125. *a. m.* Adult mouth. *cl. sp.* Clavate spines. *dent.* Tooth. *l. a.* Stumps of larval arms. *o. l.* Remnant of oral lobe of larva.

Fig. 17.—Oral view ⎱ of imago which has lived for some days after
Fig. 18.—Aboral view ⎰ the metamorphosis. × 125. *sph.* Sphæridum.

Chromosomes, Heredity and Sex: A Review of the Present State of the Evidence with regard to the Material Basis of Hereditary Transmission and Sex-Determination.

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With 4 Text-figures.

It is now rather over ten years since the hypothesis was first put forward that the segregation of Mendelian allelomorphic characters is caused by the pairing in "synapsis" of chromosomes of paternal and maternal origin, and their subsequent separation into different gametes (**58, 59, 64**). At about the same time, the discovery of an "accessory chromosome" in the spermatocytes of several Orthopterous and Hemipterous insects, which passes undivided into half the spermatids, led to the suggestion that this body was a sex-determiner (**31**), and shortly afterwards Castle (**13**) put forward the hypothesis that sex-determination is due to a pair of Mendelian factors, which segregate in gametogenesis in such a way that every germ-cell bears one or other. In this paper it is not proposed to review the earlier work, nor to give an account of all the papers which have added to our knowledge of the subject, but to summarise some of the more important lines of evidence which have become available in the last few years, so as to show as far as possible the present state of our knowledge.[1] Further, it is not proposed to deal at all completely with the various theoretical speculations to which the matter has given rise, except in so far as is necessary for the

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[1] References are given on p. 517 to papers which may be regarded as typical.

understanding of the way in which the facts referred to are related to the general question of the material basis of hereditary transmission and of sex-determination.

The argument falls naturally into the two divisions of the relation of the chromosomes to Mendelian heredity on the one hand and to sex on the other, but these two are intimately connected by the phenomena of sex-limited inheritance, from cases of which some of the most important evidence with regard to both branches has been obtained. It will, therefore, be most convenient to consider in their relation to chromosome behaviour, first, heredity alone, then sex, and finally the phenomena of sex-limited inheritance.

(1) Chromosomes and Mendelian "Factors."

The opinion that the chromosomes are the "bearers" of characters which show Mendelian inheritance has been supported on several quite distinct grounds, some of which are inferential, others depending on direct observation. The earlier arguments in favour of this idea were founded entirely on observations which suggested that the behaviour of the chromosomes in the maturation of the gametes is of exactly the kind which would give rise to Mendelian segregation. The facts are so familiar that no detailed account is needed. It has been maintained by many observers, especially in cases in which the chromosomes differ conspicuously among themselves in size, that the nuclei of the somatic cells and spermatogonia or oögonia contain a double complement, composed of sets of maternal and paternal origin respectively, and that in "synapsis" each maternal chromosome first pairs with a corresponding paternal one, and then separates from it at one of the maturation divisions into a different daughter-cell. If, then, each chromosome corresponded with a Mendelian character, and if chromosomes bearing allelomorphic characters always paired together, the segregation of the members of an allelomorphic pair into different gametes would be accounted for. And since it is a matter of chance with regard to any pair whether the paternal or maternal

element goes to a particular daughter-cell, the independence of different allelomorphic pairs in the formation of gametes is also explained. The mechanism for producing Mendelian segregation appears so perfect that it is difficult to believe that the two phenomena are unrelated, but there are serious difficulties which have first to be explained. The first of these is that there may be more pairs of allelomorphic characters than there are pairs of chromosomes, and yet the characters of different pairs show no constant association with one another. Several theoretical suggestions have been made to meet this objection, most of which assume that the chromosomes are not indivisible units, but are made up of smaller parts, each of which is the bearer of one Mendelian character. By some it is supposed that the units may be interchanged during synapsis; by others, that they become separate in the " resting " nucleus, and that it is a matter of chance whether they return into one or other of the homologous chromosomes to which they belong. The evidence for the compound nature of chromosomes is now so strong[1] that the difficulty cannot be regarded as very serious, but the exact manner in which the units are arranged in the chromosomes is far from being settled. One hypothesis with regard to this part of the question will be discussed more fully later, in connection with gametic coupling.

The second objection to the hypothesis that the pairing and separation of chromosomes in gametogenesis gives rise to Mendelian segregation is more serious, since it is based on the denial that the chromosomes behave as described. Some observers refuse to credit the chromosomes with individuality of any kind (33), and without some sort of individuality,

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[1] Compound chromosomes have been described in Ascaris (T. Boveri, ' Ergebnisse über die Konstitution der chromatischen Substanz des Zellkerns,' Jena, 1904, p. 27); in the bee (H. Nachtsheim, ' Arch. Zellforsch.,' xi, 1913, p. 169) and in other cases. The writer finds that in the nearly related moths, Nyssia zonaria and Biston hirtaria, the chromosomes of the former are exactly four times as numerous as in the latter, and much smaller, so suggesting that those of B. hirtaria may be compound.

leading to constancy of behaviour of the hypothetical units which "bear" the Mendelian factors, the whole hypothesis would fall to the ground. Others, while admitting the conjugation of chromosomes in synapsis, maintain that it is not a mere coming together in pairs, followed by complete separation, but that the two chromosomes which pair fuse so intimately as to make separation of the parts almost or quite impossible (**10, 10a**). Others, again, deny the existence of a "reduction division" in Weismann's sense, and maintain that parts of both the paternal and maternal chromosomes go into all the gametes (**32**). If any of these conditions were general it would completely destroy the basis of the hypothesis under consideration. When, however, the various objections are critically examined, they begin to appear less fatal to the hypothesis. In the first place, the three classes of objection mentioned are mutually exclusive; if the supposed facts on which any one of them is founded are genuine, the others must of necessity be mistaken. Secondly, they are largely founded on negative evidence, and if an observer, however competent, fails to find certain phenomena in the material on which he is working, this not only does not prove that the phenomena do not occur in other cases, but it may not even prove that they do not occur in his own material. The ease with which the alleged phenomena are observed varies greatly in different species, and they may quite possibly occur in cases in which the nature of the material makes proof or disproof of their occurrence impossible. In the opinion of the writer the wide-spread agreement among workers on chromosomes in favour of the existence of some kind of individuality, and of a genuine reduction division, makes the first and third of the objections mentioned of small importance. With regard to the complete separation of chromosomes which have paired in synapsis there is much less unanimity, and the question must be regarded as still open.[1]

[1] In this connection, the discussion by W. E. Agar, 'Quart. Journ. Micr. Sci.,' 57, 1911, p. 1, based on peculiarly favourable material (Lepidosiren), is of importance.

Apart from the fact that, according to many observers, the pairing and separation of the chromosomes in the maturation divisions provide just the mechanism required to bring about Mendelian segregation, there are two other lines of argument which point in the same direction. One of these is again inferential only, the other depends on direct observation. The former is concerned with the phenomenon of gametic coupling—the fact that in a number of cases two separate Mendelian units tend to be more or less closely associated in their inheritance. Gametic coupling was first discovered by Bateson and Punnett (**6, 7**) in plants. In animals it has been worked out rather fully by Morgan in Drosophila (**41**, etc.), and is now known to be widely distributed. The facts, put shortly, are that if a form possessing characters A and B is mated with one from which these factors are absent (represented a, b), the crossed individual produces gametes, most of which bear either both A and B, or neither of them, the combinations A b and a B being relatively rare. The fact that there is this association in transmission between distinct Mendelian factors is strong evidence that they are borne in some body which behaves in gametogenesis as a unit, and the only bodies known to behave in the way required are the chromosomes. This argument is strengthened by the behaviour of such characters in cases of sex-limited inheritance, which it will be convenient to discuss at a later stage. It is also strongly supported by the fact that in Drosophila there are three groups of such coupled characters; each of the characters included in any one group shows coupling with the others of the same group, but characters belonging to different groups are inherited independently of one another. Morgan has put forward a hypothesis which it must be admitted is rather speculative, to account for these facts (**42-44**). He suggests that the factors for the coupled characters are all borne in one chromosome, the chromosome being regarded as consisting of a series of units arranged along its length like beads on a string, each unit bearing one "factor." In synapsis two such chromosomes pair side by

side, corresponding units being opposite one another. Several
observers (**10a, 28, 29**) have described chromosomes as
twisted round each other in pairing, and Morgan suggests
that if, when they are so twisted, the split which separates
the chromosomes is straight, as Jannsens has maintained (**26**),
the resulting daughter-chromosomes will not be identical
with those which paired, but will consist of parts of each.
When the split occurs, units which are arranged next to each
other will usually go into the same half, but units which are
widely separated will often go into different halves. In this
way he accounts for the different degrees of coupling which
are found between different characters in the same species.
The twisting of the chromosomes round each other in synapsis
appears undoubtedly to occur in certain cases, but until the
splitting across the twist postulated by Morgan has been
shown with certainty to occur, his hypothesis must be
regarded as almost entirely speculative.

The last class of evidence with regard to the relation of
chromosomes to Mendelian factors depends on direct observa-
tion. At present few cases are known which bear directly on the
question, and only two will be mentioned at this point. First
may be mentioned the work of Federley (**20**). He found that
hybrids between moths of the genus Pygæra showed that
certain features of one species were dominant, while in other
respects the hybrids were intermediate. When the hybrid was
mated with one of the parent species, in most respects the off-
spring were all again hybrid in character; in one or two
features, however, segregation took place. On investigating
the behaviour of the chromosomes, he found that in the
spermatogenesis of the hybrid nearly all the chromosomes
failed to pair; the spermatocyte chromosomes were almost
of the somatic number, and divided equationally in both
divisions. One or two chromosomes, however, paired and
segregated normally. When the hybrid P. *curtula* ♂ × P.
anachoreta ♀ was paired back with pure anachoreta ♀
the offspring contained almost a complete triple set of
chromosomes, for the hybrid provided almost complete

curtula and anachoreta sets, and the anachoreta parent a (haploid) set of anachoreta. The secondary hybrid therefore contained a double (diploid) set of anachoreta, and a haploid set of curtula chromosomes. In this case, in spermatogenesis of the secondary hybrid, normal pairing took place between the anachoreta chromosomes, while, as in the primary hybrid, the curtula chromosomes divided equationally, with the result that the gametes of the secondary hybrid contained a haploid curtula + a haploid anachoreta set. If, now, the chromosomes are the bearers of Mendelian factors, the fact is explained that the primary hybrid, when paired back with curtula, shows in

TEXT-FIG. 1.
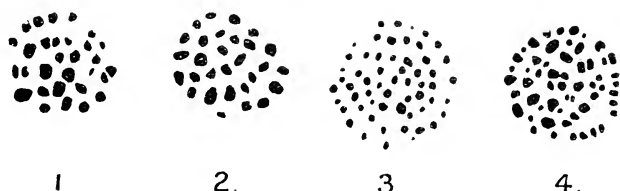
1. First spermatocyte equatorial plate of Pygæra anachoreta. 30 chromosomes. 2. Similar equatorial plate of P. curtula, 29 chromosomes. 3. First spermatocyte equatorial plate of hybrid P. anachoreta ♀ × P. curtula ♂; 58 chromosomes, so that only two chromosomes have united to form a pair. All the others are about half the size of the spermatocyte chromosomes of the pure species. 4. First spermatocyte equatorial plate of secondary hybrid, P. anachoreta ♀ × (P. anachoreta ♀ × P. curtula ♂); 56 chromosomes, of which about 30 are large, and consist of two units paired together (presumably anachoreta chromosomes) and about 26 are small, unpaired, presumably curtula chromosomes. (After Federley.)

general no segregation, since in each generation nearly complete haploid sets of chromosomes of both species are present in the gametes. One or two chromosomes, however, pair and separate, and correspondingly one character at least was observed which showed Mendelian segregation.[1]

[1] The present writer has confirmed Federley's results as to the behaviour of the chromosomes, in reciprocal crosses between Biston

The second piece of direct evidence connecting chromosomes with inherited characters is due to Baltzer (**5**). Previous investigators had found that the cross between the sea-urchins Sphærechinus granularis ♀ and Strongylocentrotus lividus ♂ produces plutei intermediate in character between those of the parent forms, while the converse cross, in the rare cases when the larvæ survive, gives plutei in which the skeleton is of the pure maternal form. Baltzer has examined the behaviour of the chromosomes in the two crosses. He finds no peculiarity in the cross with Sphærechinus ♀, but when Strongylocentrotus is used as the female parent, he finds that about 16 chromosomes are constantly omitted from the daughter-nuclei at the first and second segmentation division of the egg. He infers by three distinct methods that the eliminated chromosomes are paternal, i.e. derived from Sphærechinus, and since the haploid number of Sphærechinus is 20, there remain only about four Sphærechinus chromosomes in the hybrid plutei, which are maternal in character, while the full number remains in the intermediate plutei produced by the converse cross. That the eliminated chromosomes are paternal is inferred (1) from the shapes and sizes of the chromosomes; (2) from a study of a tetraster, in which the number corresponds with the expectation if two spermatozoa had entered the egg and only eight of the sperm-chromosomes behaved normally; (3) by the fertilisation of non-nucleated Strongylocentrotus egg-fragments with Sphærechinus sperm, in which again only about four chromosomes divide normally.

Tennent (**60**) similarly finds elimination of chromosomes in the cross Hipponoë ♀ × Toxopneustes ♂, but none in the converse cross. In this case the Hipponoë characters dominate in the pluteus whichever way the cross is made, but since the Hipponoë chromosomes are presumably present

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hirtaria and Nyssia zonaria. In this case the hybrids are sterile, so the later generations are not available. The work is to be published in the next number of the 'Journal of Genetics.'

in both the reciprocal crosses, this result would be expected if the Hipponoë characters were dominant.[1]

Indications of the same sort, but somewhat less direct, have been given by Herbst (**25**). He has found in occasional hybrids between Sphærechinus ♀ and Strongylocen-trotus ♂, individuals with purely Sphærechinus skeleton on one side, and intermediate skeleton on the other. On the side with Sphærechinus skeleton the nuclei have half the volume of those on the other side, indicating that they contain fewer chromosomes, and he supposes that the sper-matozoon has conjugated with one of the nuclei of the first two blastomeres, so that half the larva has purely maternal, the other half hybrid nuclei.

Evidence of this kind, while not proving that the chromo-somes are directly concerned in the transmission of inherited characters, makes such a hypothesis very plausible.[2] Much unnecessary confusion, however, has arisen, from stating the hypothesis in the form—" the chromosomes are probably the bearers of inherited characters." Evidence has been adduced that the cytoplasm plays some part in determining these characters, and it has therefore been maintained that the statement is disproved. No one, however, would suppose that the chromosomes could act alone; they must act in and by their relation with the cytoplasm, and if the cytoplasm is that of a different species, the total effect must necessarily be different. A simple chemical analogy will make this clear. The substance represented CH_3H is a hydrocarbon; exchange

[1] The writer and J. Gray (' Quart. Journ. Micr. Sci.,' 58, 1913, p. 483) found elimination of one or two chromosomes in the cross Echinus miliaris ♀ × E. acutus ♂, but none in the converse cross. In the case of the cross E. acutus ♀ × E. esculentus ♂, in which some chromosomes are lost by becoming vesicular and failing to divide, there is evidence that the vesicular chromosomes are maternal (J. Gray, ' Quart. Journ. Micr. Sci.,' 58, 1913, p. 447). In these cases it was not found possible to correlate the chromosome behaviour with the inherited characters of the plutei.

[2] The evidence adduced by Goldschmidt (' Arch. Zellforsch.,' ix, 1912, p. 331) has not been mentioned, since its correctness has been disputed (O. Renner, ' Berichte d. deutsch. Botan. Gesellsch.,' xxxi, 1913, p. 334).

the hydrogen atom for chlorine and the substance CH_3Cl has different properties, while those of CH_3OH are different again. An exactly corresponding series, in composition and properties, is provided by the substances C_2H_5H, C_2H_5Cl, C_2H_5OH, and it would not be regarded as exaggeration to say that the change from a hydrocarbon to a chloride or an alcohol is produced by replacing the hydrogen atom by another atom or radicle. But the various atoms which may replace the hydrogen do not have exactly the same effects when attached to C_2H_5- as when attached to CH_3-; their effects are similar, but not identical. So when a chromosome of species A is replaced by one of species B, the effects of B in A cytoplasm cannot be expected to be identical with those produced in its natural environment. And when, as in Godlewski's famous experiment, it was found that Antedon chromosomes appeared to have no effect in Echinus eggs, it may well be that the environment was too strange for any interaction to be possible, just as the replacement of hydrogen by chlorine in some compounds is easy, in others difficult or impossible.

Further evidence with regard to the functions of the chromosomes in heredity will be given when dealing with sex-limited inheritance, after the general question of sex-determination has been considered.

(2) Sex.

The evidence of connection between chromosomes and sex-determination is on the whole more complete than that which connects chromosomes with Mendelian factors. Here again the bulk of the evidence is indirect rather than direct, but since there are only two sexes it is much easier to associate a particular chromosome with sex-determination than to show that any one is connected with the transmission of a particular Mendelian unit. The evidence that a particular chromosome in many cases invariably accompanies one sex is so clear that it may almost be regarded as direct evidence for sex-determination by that chromosome, and some of the facts

are of such a nature that it would hardly be exaggeration to claim them as proving that hypothesis.

Very early in the present century cases were observed in insects in which one chromosome differed conspicuously from the rest in spermatogenesis, especially in taking no part in the spireme stage, but behaving as a "chromatin-nucleolus." It was then found that these species had an odd number of chromosomes in the male, an even number in the female, and that the difference was due to the presence of a pair of such "accessory" chromosomes in the female, and only one in the male. The "accessory" in the male has no mate, and goes over undivided in one (usually the first) spermatocyte division, but divides equationally in the other, with the result that of the four spermatids derived from each spermatogonium, two possess it and two are without it. In the female the two accessories pair and separate, so that one is present in every egg-nucleus. If all the spermatozoa are equally functional, half the zygotes will have one accessory, the other half two, and the natural inference was drawn that the former become males, the latter females. Such cases were at first known only in Orthoptera and Hemiptera; they have now been observed in most orders of insects except Lepidoptera and Hymenoptera, and in many other groups, a list of which is given later. Not all species of these groups, however, show this difference between the sexes, and doubt was cast on the sex-determining function of the accessory chromosome on the ground that its existence should be expected in every species which has two sexes. The next step was made by Wilson (65). He found that in certain Hemiptera the spermatogonia contained two "accessories," one of which was noticeably larger than the other, and that in consequence half the spermatids contained a large one, half a small, but that the females of these species had two large ones, so that each mature egg contains one. To avoid confusion he named these unequally paired bodies "idio-chromosomes," and the unpaired body of the species previously described, a "heterotropic" chromosome. He further dis-

covered that in a series of species all stages could be found
between cases in which both sexes had equally paired idio-
chromosomes, through the condition in which they are
unequally paired in the male, to the extreme case of total
absence of the smaller one in the male. It therefore seemed
natural to conclude that whether they were in appearance
alike or unlike in the male, they were different in function,
and that, since one of them showed all stages of disappearance
in related species, this one is probably not functional as a
sex-determiner, even when it is present. Two main hypotheses
have been suggested to explain their action. Wilson first
suggested[1] that the two similar idiochromosomes of the
female bear respectively male and female determiners, that
the single functional one of the male bears a male determiner,
and that selective fertilisation occurs in such a way that
male-bearing spermatozoa fertilise female-bearing eggs, and
spermatozoa without sex-factor fertilise male-bearing eggs,
thus :
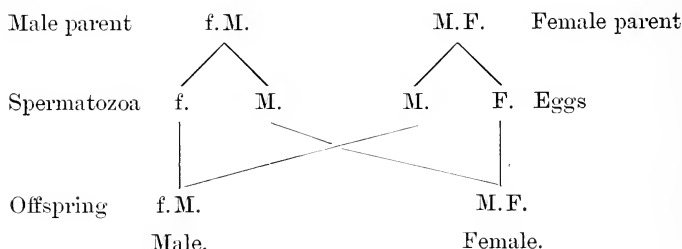
Femaleness is supposed to be dominant over maleness, and
therefore the zygote MF is a female, Mf a male (f repre-
senting absence of sex-determiner). A second hypothesis,
now more generally adopted, is due to Wilson and Castle (**67,
14**), and is accepted by Morgan, who seems to have arrived at
it independently : it is that sex is not due to specific male and
female factors, but to the presence of one factor in greater or
less amount. In the cases described, a " single dose " of this

[1] E. B. Wilson, ' Journ. Exp. Zool.,' iii, 1906, p. 29. The same
suggestion was made on independent grounds by the writer (' Proc.
Zool. Soc.,' 1906, p. 132, and ' Proc. Roy. Soc.,' B, lxxxii, 1910, p. 106).
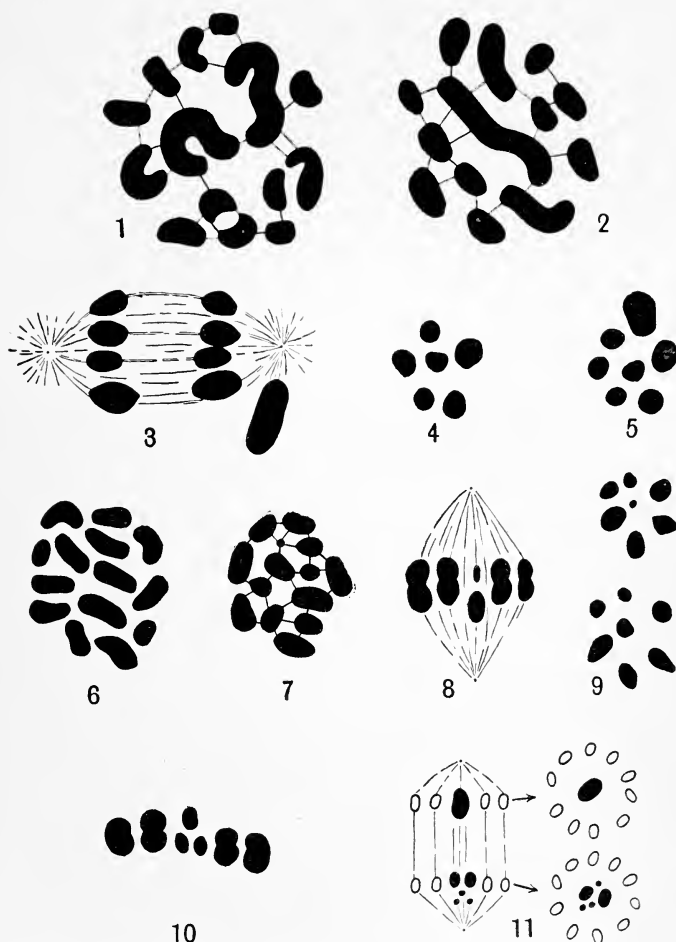
Forms of heterochromosomes in various Hemiptera. 1–5.
Protenor belfragei (from Wilson). (1) Oögonial equa-
torial plate ; two large heterochromosomes. (2) Spermatogonial
equatorial plate ; one large heterochromosome. (3) Anaphase,
second spermatocyte division, side view ; large heterochro-
mosome below the right pole. (4, 5) Second spermatocyte divi-
sion, polar view of two daughter-plates ; heterochromosome in
group 5. 6–9. Euschistus variolarius (from Wilson).
(6) Oögonial equatorial plate ; no conspicuously small chromo-
some. (7) Spermatogonial equatorial plate ; one very small
idiochromosome. (8) Second spermatocyte metaphase ; large
and small idiochromosomes in centre. (9) Sister groups,
second spermatocyte division ; small idiochromosome in upper,
large in lower. 10 (from Wilson). Rocconota annuli-
cornis, metaphase of second spermatocyte, showing large idio-
chromosome paired with two small. 11 (from Wilson, after
Payne). Acholla multispinosa, second spermatocyte ana-
phase, showing large idiochromosome paired with five small.

factor produces maleness, a double dose femaleness. The second hypothesis is preferable in not involving selective fertilisation, but involves certain other difficulties from which the former escapes.

Essentially similar phenomena with regard to sex-chromosomes have now been described not only in insects, but also in Myriapods (9), Arachnids (8, 62), Molluscs (70), Nematodes (11, 23, 45, 51), Birds (24, 24a), and Mammals (27, 56, 68, 69). In all these cases the male is described as having a deficiency of one (or sometimes two) chromosomes, or as having a small one in place of a corresponding large one in the female.

There are also a number of complications which have been observed in various cases, which will only be referred to shortly. In some species the male is described as having two or more chromosomes less than the female (66, 47, 48) ; in this case the two odd ones travel together into the same daughter-cell in the spermatocyte divisions, so that they may be regarded as behaving as one chromosome divided into two parts. In some cases both members of the pair may be compound. This class of facts is possibly of importance in connection with sex-limited inheritance. Cases of peculiar behaviour in hermaphrodite species have also been described, the true nature of which is at present obscure.[1] Two examples, however, must be given, one of a hermaphrodite, the other of a parthenogenetic species, since these have been worked out rather fully and add very important facts to our knowledge. The chromosome-cycle of the Nematode Rhabdonema (Rhabditis) nigrovenosum has been investigated independently by Boveri (11) and Schleip (51), from whose papers the following account is combined. The species, as is well known, has alternate generations which are hermaphrodite and bisexual. The hermaphrodites have as diploid number twelve chromosomes ; their primary oöcytes have six

[1] E. g. in Pteropoda, B. Zarnik, 'Verhandl. Deutsch. Zool. Gesellsch.,' 1911; summarised by Schleip, ' Ergebn. und Fortschritte der Zool.,' iii, 1912, p. 250.

TEXT-FIG. 3.
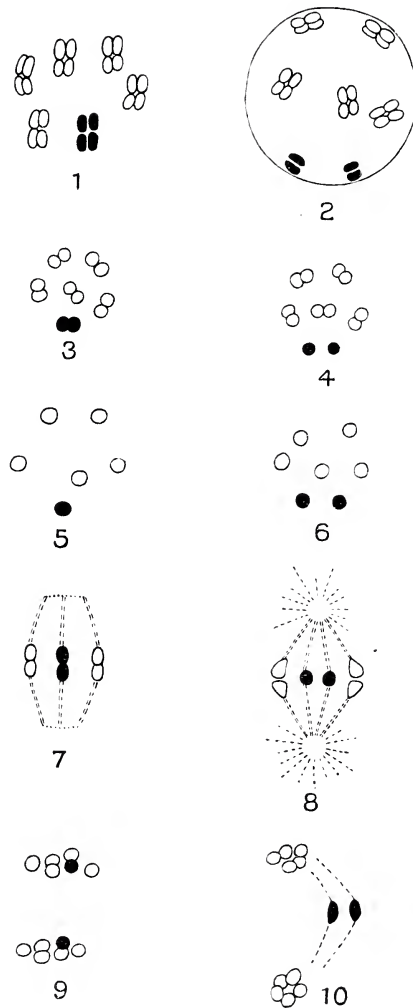
Oögenesis and spermatogenesis of the hermaphrodite generation
of Rhabdonema nigrovenosum. (After Boveri.) Oöge-
nesis on left, spermatogenesis on right. Heterochromosomes
black. (1) and (2) Primary oöcyte and spermatocyte prophases;
(3) and (4) daughter-groups of first maturation division; (5) and
(6) equatorial plates of second maturation division; (7) and (8)
diagrammatic side views of same stage; (9) and (10) anaphases
of second maturation division. According to Schleip one of
the heterochromosomes is included in one daughter-group, the
second is left out entirely.

doubles, the mature egg six singles. The spermatogonia have twelve, the spermatocytes five doubles and two singles; the singles do not divide at the second spermatocyte division, and one of them gets left on the spindle, so that half the spermatids have six, half have five.[1] Half the fertilised eggs thus have twelve, and become females ; half have eleven, and become males.   In the gametogenesis of the free-living bisexual forms, all mature eggs contain six ; in the males, the odd one does not divide at one spermatocyte division, so that half the spermatids have six and half five.   Those with five appear to degenerate, so that all functional spermatozoa have six, and thus all fertilised eggs have twelve and become hermaphrodites.   Why, in one generation, individuals with twelve become females, in the next, hermaphrodites, is not explained.   It is noteworthy that the absence of one chromosome is not the cause of the production of spermatozoa rather than ova, since both oögonia and spermatogonia of the hermaphrodite have twelve.

The second special case is that of the Aphids and Phylloxerans described by Morgan (**35–37**) and von Baehr (**3**). Morgan's account of Phylloxera caryaecaulis may be taken as an example.   The "stem-mother" has six chromosomes; her parthenogenetic eggs have no reduction and contain six.   The parthenogenetic females produced from these eggs are of two kinds, which may be called female-producers and male-producers.   Each form contains six chromosomes, but the male-producer has five large and one small, the female producer six large.   Morgan suggests, without actually proving it, that the cause of this is that one of the chromosomes is compound, and part of it is extruded without division at the polar mitosis of the egg which gives rise to a male producer.[2]   The male-producing female lays small eggs which

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[1] This is Schleip's account of the second spermatocyte division; according to Boveri either both the "accessories" may be included in one spermatid or one may go into each. thus giving spermatids either with five and seven, or with six chromosomes.

[2] In ‘ Heredity and Sex ’ (New York, 1913, p. 182), Morgan suggests that the difference between female-producer and male-producer may be

develop into males ; these extrude one chromosome undivided
at the single polar division, so that males have five chromo-
somes.   Sexual females are produced from large eggs which
have a normal equation division, and therefore contain six.
In spermatogenesis two kinds of spermatids are produced,
one with three, the other with two chromosomes ; the latter
degenerate, so that all functional spermatozoa have three.
The fertilisable eggs have a normal reduction, so that the
female pronucleus contains three, and the fertilised egg,
therefore, contains six, and completes the cycle by giving rise
to a "stem-mother."

The whole scheme is made clearer by the diagram on p. 504,
in which the "sex-chromosomes" are represented by X.
The difference between the chromosomes of female-producer
and male-producer is not represented, since it has not yet
been satisfactorily elucidated.   The word "egg" refers to the
egg before maturation ; in the parthenogenetic generations
the chromosome group of the mature egg is, of course, the
same as that of the individual which develops from it.

Two points of great importance should be noticed in con-
nection with these observations.   Firstly, it is possible for a
polar division to occur in such a way that one chromosome is
always thrown out with the polar body (male-producing eggs) ;
it is, therefore, not entirely a matter of chance to which end
of the spindle an undivided chromosome shall go.   This may
be a fact of very great moment in interpreting some other
cases.   Secondly, the absence of one X chromosome is not
the ultimate cause of male-production, since it is predeter-
mined in some way that some eggs shall extrude X (possibly
by the loss of one portion of a compound chromosome in the
previous generation), and that other eggs shall not.   But this
cannot logically be regarded as a proof that the presence or
absence of X is not the cause of femaleness or maleness ; it
only means that some factor is present which decides whether

referred back to the stem-mother ; there would be two kinds of stem-
mothers, one of which produces female-producing offspring, the other
male-producing.

Stem-mother $4 + 2\,X$

Egg $4 + 2\,X$ Egg $4 + 2\,X$

Polar body $4 + 2\,X$ Polar body $4 + 2\,X$

Female-producer $4 + 2\,X$ Male-producer $4 + 2\,X$

Large egg $4 + 2\,X$ Small egg $4 + 2\,X$

Polar body $4 + 2\,X$ Polar body $4 + X + X$

Sexual female $4 + 2\,X$ Male $4 + X$

Egg $2\,(2 + X)$ Primary spermatocyte $2 \times 2 + X$

1st polar body $2 + X$

2nd polar body   Mature egg   Functional   Degenerate
$2 + X$ spermatid   spermatid 2

$2 + X$ $\times$ $2 + X$

Stem-mother $4 + 2\,X$
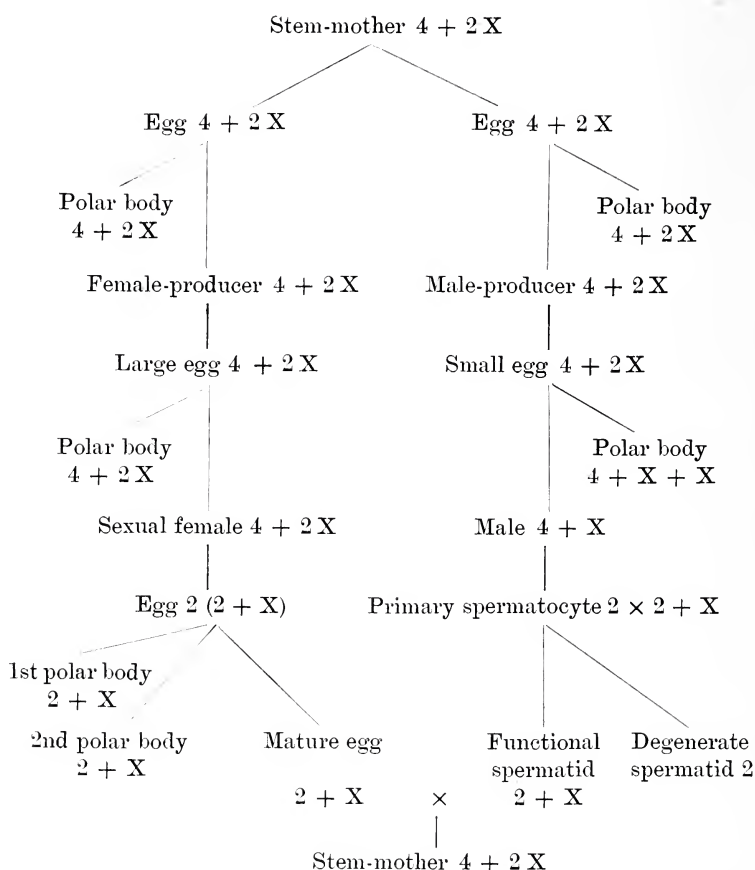
X shall be extruded or not. So much emphasis has been laid on these facts by the opponents of the sex-chromosome hypothesis that a simple analogy may perhaps be forgiven. If in searching for the cause of a disease a pathologist found a bacterium, inoculation with which always produced the disease, he would be justified in assuming provisionally that this bacterium was its " cause." It would be no argument against his hypothesis to say that the " cause" was the injection-syringe, or the experimenter, both of which, however, may have to act before the cause can take effect.

In all the examples mentioned hitherto, the female has had

two similar "sex-chromosomes," the male one like those of the female and one smaller or absent. The facts of sex-limited inheritance make it probable that in some groups the converse arrangement should be found, and two cases of this have already been described, while the writer has a third at present under investigation. The first account of unequally paired chromosomes in the female was that of Baltzer in the sea-urchins Strongylocentrotus and Echinus (**4**), in which he describes a hook or horse-shoe-shaped element in half the eggs, replaced by a rod in the other half. Tennent (**60**), however, on similar evidence, concludes that in American species of Hipponoë and Toxopneustes it is the male in which there is an unpaired chromosome, and those who have had experience of detailed study of Echinoid chromosomes will regard the question as still open until further confirmation is forthcoming.

Recently an unequally paired chromosome in the female has been described in Lepidoptera, an order in which the probability of its existence had been predicted on the ground that sex-limited transmission occurs in the female. Seiler (**52**) describes in the eggs of the moth Phragmatobia fuliginosa a large chromosome which in one equatorial plate of the polar mitoses divides into two and in the other remains single. It may divide in either the inner or the outer plate. Since there are 27 ordinary chromosomes, the mature egg may contain either 28 or 29. All spermatocytes have 28. Since the equatorial plate of the first polar spindle has 28, there is no evidence to show whether the eggs which have the divided chromosome give rise to males or to females. Seiler inclines to the view that eggs with 29 give rise to females, but it is possible that both the large chromosomes of the male are really compound, and that only one is compound in the female.

The present writer has now under investigation a somewhat similar case in Abraxas grossulariata. In normal females of this species the oögonia have 56 chromosomes, but in a strain in which abnormal sex-ratios appear there are 55

(**17, 18**). In the eggs of females of the strain with 55 there are 28 in one equatorial plate of the second polar division, 27 in the other.[1] Since the females of this strain have 55 (diploid number),[2] and since all spermatozoa almost certainly

TEXT-FIG. 4.

Outer   Inner
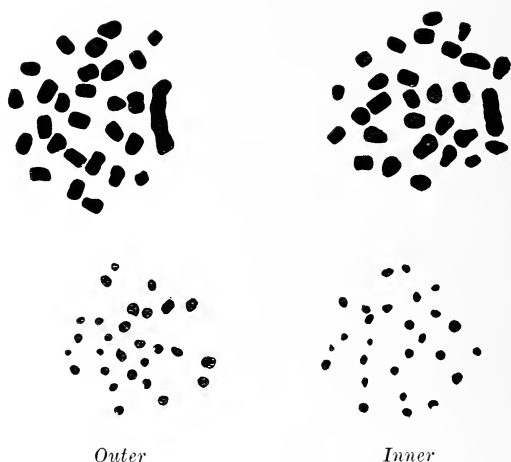
Two upper chromosome groups, Phragmatobia fuliginosa, after Seiler: On the left, outer polar plate, 27 ordinary and one large chromosome (total 28). On the right, inner polar plate, which gives rise to egg nucleus, 27 ordinary and one large chromosome divided (total 29). Each figure was reconstructed from two sections. Two lower chromosome groups, Abraxas grossulariata: On the left, outer polar plate, 28 chromosomes; on the right, inner polar plate with 27. The two groups are separated by one section which contains no chromosomes; all the chromosomes of each group are in one section.

[1] There are certain points in connection with this case which still require further investigation, but that in eggs of this strain one polar plate has 27, the other 28, appears to be incontestible. In females not belonging to the strain referred to, both polar plates have 28, as would be expected from the oögonial number, 56.

[2] The exception in family 12·25, mentioned in the paper referred to, will be discussed in the full paper; no other exception to the number 55 was found in over forty larvæ of this strain in which oögonial figures could be counted.

have 28, it can hardly be doubted that eggs with 27 give rise to females, those with 28 to males. This case is thus exactly the converse of the typical examples of an "accessory" chromosome in the male, as seen in the Hemiptera, Orthoptera, etc. It is remarkable that in this case the male has one chromosome more than the female, although in forms in which the spermatozoa are dimorphic the male has one less. The bearing of this fact on theories of sex-determination will be referred to again later.

Finally, mention must be made of the conditions found in the Hymenoptera. In this order parthenogenesis is common, but differs from that of most parthenogentic animals in the existence of two polar divisions. Most commonly unfertilised eggs of Hymenoptera give rise to males; when females are produced parthenogenetically there is evidence that no reduction occurs, either because one or both polar divisions are suppressed,[1] or because both are equational. No case is known in which males are produced from eggs which are certainly fertilised, and in all which have been examined the male has half the number of chromosomes found in the female. The male has a haploid set, the female a diploid. Correspondingly, in all cases examined,[2] one maturation division of the spermatocytes is suppressed, so that the spermatocytes contain the same (haploid) number as the spermatogonia.[3] Nachtsheim (**46**) brings these phenomena into line with the facts known about sex-chromosomes by the suggestion that

[1] L. Doncaster, "Gametogenesis of the Gall-fly Neuroterus lenticularis," 'Proc. Roy. Soc.,' B, lxxxiii, 1911, p. 476. It should be noted that in Neuroterus, as in Morgan's case of Phylloxera, it is predetermined which eggs shall undergo reduction and become males, and which eggs shall suppress the maturation divisions and become females, since all the eggs laid by any one female behave in the same way.

[2] E.g. in the bee, F. Meves, 'Arch mikr. Anat.,' lxx, 1907, p. 414; H. Nachtsheim, 'Arch. f. Zellforsch.,' xi, 1913, p. 169; in Neuroterus, L. Doncaster, 'Proc. Roy. Soc.,' B, lxxxii, 1910, p. 88. Several other forms give concordant results.

[3] Armbruster, L., 'Arch. f. Zellforsch.,' xi, 1913, p. 242, interprets his observations on Osmia differently; his figures and discussion are not convincing.

two of the chromosomes of the female are sex-chromosomes. The male, with half the number, has only one, but since all spermatozoa bear it, all fertilised eggs have two, and so become females. Similar suggestions had previously been made by Wilson and others.

In this connection mention should be made of the conditions found in Rotifers, about which there has been some controversy, but the facts appear fairly clear. In Hydatina, Lenssen (**30**) has shown that in the parthenogenetic eggs which will become females one polar division is suppressed, while it takes place normally in male-producing eggs and in eggs which will be fertilised ("Dauereier"). He believed that female-producing parthenogenetic eggs produced no polar body, male-producing eggs and Dauereier, one. Whitney (**63**), however, has shown that female-producing eggs have one polar body, male-producing eggs two. The present writer has confirmed Lenssen's observations on the suppression of a polar division while the egg is in the oviduct,[1] but all the observations can be brought into line by assuming that in female-producing eggs the first polar division is suppressed, but that the second takes place normally after the egg is laid, and that in male-producing eggs the first takes place before, the second after laying. In Rotifers, therefore, as in Hymenoptera, the female probably has the diploid, the male the haploid number of chromosomes.

This completes the main lines of evidence connecting sex-determination with chromosome behaviour, apart from those derived from the facts of sex-limited transmission.

(3) SEX-LIMITED INHERITANCE AND CHROMOSOMES.

Sex-limited inheritance may be defined as the transmission by individuals of one sex of a factor only or almost exclusively to offspring of the other sex. In different groups it occurs in different sexes. In the first cases to be discovered (Abraxas, canary, fowl) the female transmits certain

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[1] Unpublished.

characters only to her sons, while the male transmits the
same characters to both sons and daughters. Later it was
found that in Diptera (Drosophila) and in Mammals (cat,
man) the male transmits characters only to his daughters,
while the female transmits them impartially. The facts
show that in one case the female, in the second the male, is
constantly heterozygous for certain features, and that in
inheritance the factors for these features are closely coupled
with a sex-determining factor. The case of Drosophila will
be the most convenient to deal with first (38–41). Morgan
finds that the male transmits certain of the normal features
of the species (red eye, long wing, brown colour) only by his
female-producing gametes, so that when mated with a female
lacking any of these characters, the male offspring are without
them. Now the male Drosophila has been shown by Miss
Stevens (55) to have a pair of unequal chromosomes, and
Morgan makes the not unnatural assumption that the larger
of these chromosomes bears not only the sex-factor, but also
the factors for the sex-limited characters. Since half the
spermatozoa contain the larger chromosome, and half lack it,
and since all the eggs contain it because it is equally paired
in the female, half the zygotes have it in duplicate and
become females, half receive it only from the mother and
become males. And since, by hypothesis, the larger idio-
chromosome of the male bears both the sex-factor and the
factors for the sex-limited characters, the latter are trans-
mitted by the male to his female offspring only. Morgan
has carried this conception further in connection with the
phenomena of gametic coupling. When a male having two
sex-limited characters is crossed with a female lacking both, the
female offspring have both, the males neither. In the game-
togenesis of the heterozygous female so produced, the two
factors are, in some cases, not distributed evenly among the
gametes, but tend to be associated, so that the gametes which
bear either both or neither are much more numerous than
those which bear one only. A diagram will make this clearer,
in which the two characters concerned are represented as A

and B, their absence as a and b, the sex-factor as X, and the absence of sex-factor by a dash ($-$).

Parents   Male   XAB $-$ $\times$ Xab Xab   Female

Offspring F_1 Male   Xab$-$ XAB Xab   Female

Gametes of F_1 $-$ Xab   nXAB XAb XaB   nXab

The number n, by which the combinations AB or ab out-number aB and Ab, varies in the case of different characters from quite small numbers to 100 or more. Now Morgan supposes that in the synapsis stage of the first-cross female (F_1), the chromosomes XAB and Xab pair together, and become twisted round one another as was mentioned earlier, and that in subsequent separation it is possible for A to exchange places with a and B with b, and that the frequency of this interchange (" crossing over ") will depend on the positions of the units representing A and B in the elongated chromosome.[1] In the male, no such " crossing over " is possible, since the chromosome XAB or Xab has no similar mate.

There is one difficulty, however, which makes this " crossing-over " hypothesis doubtful, in spite of its beautiful simplicity. It is that exceptions to sex-limited transmission occur in almost all the known cases. Morgan himself has recorded some in Drosophila, but prefers to regard them as due to experimental error. In other cases, however, they are indubitable. If sex-limited transmission were due to the existence in an unpaired chromosome of the units determining the characters, those characters must always without exception be transmitted from the male to the female, or, in the Lepidoptera-bird group, from the female to the male. Exceptions, however, always occur, with greater or less frequency

[1] This idea has been worked out in some detail in the case of Drosophila by A. H. Sturtevant, ' Journ. Exp. Zool.,' xiv, 1913, p. 43.

in different cases, and another explanation, equally concordant with the known facts of chromosome behaviour, is available. It has been shown by several observers (**66, 47**) that the "sex-chromosome" is not infrequently compound, and in one case (**49**) the two parts are constantly separate, though they are reported always to go to the same pole of the spindle. If now one portion of the "sex-chromosome" bears the sex-factor, the other portion the sex-limited factors, failure of sex-limited transmission will arise whenever the components of the chromosome become separate, and go to different poles. The frequency with which this happens may be expected to vary in different cases. This suggestion, due originally to Wilson (**66**), really differs from Morgan's only in the supposition that the sex-chromosome is commonly coupled with a chromosome which bears the sex-limited factors, instead of assuming that these factors are borne in the sex-chromosome itself. The exceptions to the normal sex-limited transmission make this latter assumption untenable.[1]

The facts of sex-limited transmission thus support the hypothesis that both ordinary Mendelian factors and the sex-determining factor or factors are borne by chromosomes, although the details of their relations are still very far from being clear.[2] That there is some intimate relation, however, between a chromosome and the transmission of sex-limited characters is almost certain from the fact that sex-

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[1] Since this was written Bridges (**12**a) has suggested a hypothesis to avoid this difficulty. He assumes that exceptions are caused by "non-disjunction" of the sex-chromosomes, so that both go into one germ-cell. This explanation, if substantiated, would account for most of the recorded exceptions, but not that of the tortoiseshell male cat, which contains both the colour-factors characteristic of the female, but must be supposed to have only one sex-chromosome.

[2] The recently published work of Miss K. Foot and Miss E. C. Strobell ('Biol. Bull.,' xxiv, 1913, p. 187) cannot be used as an argument against this proposition. They have shown (as was previously known in birds and moths) that a secondary sexual character in Hemiptera can be transmitted through the sex which does not show it, but the character was not sex-limited in transmission; their results, therefore, have no bearing in the present discussion.

limited transmission by the male is only known in groups (Diptera, mammals) in which unpaired or unequally paired chromosomes have been found in the male, while of the two groups (Lepidoptera, Birds) in which sex-limited transmission by the female occurs, in one (Lepidoptera) two cases have now been described of an odd element in the female. It can hardly be coincidence that the spermatozoa should be dimorphic in respect of a chromosome in the forms in which sex-limited transmission by the male takes place, and the eggs diamorphic in the same way in those in which sex-limited transmission is by the female. It should be noted also that the unpaired chromosome has been described as compound in mammals, and one of the unequal elements as compound in Phragmatobia (moth) and possibly so in Drosophila, so supporting the suggestion that sex-limited inheritance is related to the association of a chromosome with the sex-chromosome.

One anomalous observation, however, must be mentioned. Guyer (**24, 24a**) has described an unpaired accessory chromosome in the male of the fowl and guinea-fowl, although only sex-limited transmission by the female is known in birds (fowl, canary, pigeon). Either his observation is mistaken,[1] or the facts are less simple than other cases would suggest. On Wilson's earlier hypothesis that both sexes are heterozygous for sex-factors, with selective fertilisation, the case causes no difficulty, although it is fatal, if substantiated, to the later hypothesis of the presence or absence of one single sex-factor. In this connection it may be mentioned that there are some isolated instances which suggest that in cases which have normal sex-limited transmission by one sex, there may be partial coupling of the same characters with a sex-factor in the other sex. Such cases are so doubtful and irregular in their appearance that they cannot be regarded as evidential at present, but if they should be found to be genuine, they would also support the hypothesis that both sexes are heterozygous.

[1] As this goes to press, it has been contradicted by A. M. Boring and R. Pearl ('Journ. Exp. Zool.,' xiv, 1914, p. 53).

In general, therefore, the facts of sex-limited inheritance support the contention of a direct relation between chromosomes and the transmission of inherited characters, although they do not as yet enable us to choose between particular theories of sex-determination.

(4) Conclusion and Summary.

The various classes of facts which have been described in the last two sections, if considered by themselves, provide strong evidence for the existence of an intimate relation between the presence or absence of a particular chromosome and the determination of sex. The case for the relation of chromosomes to Mendelian factors is less conclusive, but if the connection between chromosomes and sex-determination be admitted, the facts of sex-limited inheritance make it almost impossible to reject the belief in a similar connection with Mendelian factors. There are, however, certain rather grave difficulties in the way of accepting the conclusion with regard to sex which demand a few words of explanation.

In the first place, if sex is constantly determined by the presence or absence of a particular chromosome, it is difficult to understand why, within one group of animals, species of one order or class give indications that such a chromosome exists only in the male, although species of another order give exactly similar evidence that it is present only in the female. This difficulty disappears if the hypothesis is adopted that both sexes are heterozygous for sex-factors ($♀ = MF$, $♂ = Mf$), and that there is selective fertilisation in such a way that M-bearing eggs are only fertilised by f-bearing spermatozoa, F-bearing eggs by M-bearing spermatozoa. It must be admitted, however, that in the absence of any direct evidence for selective fertilisation, this hypothesis is very speculative. A suggestion which is perhaps more probable is that there are two independent sex-factors, M and F, and that when both factors are homozygous (MMFF), in some forms M is epistatic over F, giving a male; in others, F is

epistatic over M, giving a female, but that either, when homozygous, is always epistatic over the other when the latter is heterozygous. In this case, in the majority of insects and in mammals the male would be represented MMFf, the female M M F F, while in Lepidoptera and birds the male would be M M F F, the female M m F F. This hypothesis is in accord with the observed fact that in many insects and some mammals the male has one heterochromosome, the female two, while in the peculiar strain of Abraxas described, it is the female which has one chromosome less than the male.

A second problem, which is really more closely connected with this one than may appear at first sight, arises from the considerable number of cases in which sex is actually or apparently altered by conditions acting on the unfertilised egg or on the developing individual. In addition to such instances as that of the crab infected with Sacculina, in which Geoffrey Smith has shown that males may so far assume female characters as to produce ova in the testis, there are many cases in which such conditions as staleness of the eggs cause alterations in the sex-ratio (usually by increasing the proportion of males) which cannot be explained by selective mortality, nor, in fact, by any known cause except a change in the sex-determining power of the eggs.

Such cases would seem to offer an almost insuperable obstacle to the belief that sex is determined by the presence or absence of a particular chromosome, and yet the facts with regard to chromosomes are too clear and definite to be disregarded on this account. It is possible, however, that suggestions towards a reconciliation can be found by taking into consideration some of the facts which are known about the relations of sex to general metabolism. G. Smith (**53**) has shown that the change from male to female secondary sexual characters in the crab infected with Sacculina is accompanied by deep-seated metabolic changes which are induced by the parasite. Steche (**54**) has brought forward somewhat similar evidence in the case of Lepidopterous larvæ. He finds that there are important metabolic differences

between the sexes, and that, when estimated by precipitin tests, there is nearly as much difference between the blood of the male and female of one species as between the bloods of the same sex of related species. Such observations as these, taken together with the fact that the secondary sexual characters of each sex can be inherited through the other sex (**61**), suggest that possibly the fundamental difference between the sexes is not a factor which directly determines whether the individual shall possess testes or ovaries, but is really a difference of metabolism.

Where differences of metabolism are found in the two sexes it has usually been assumed that these are caused by the presence of testes or ovaries, by means of hormones or other internal secretions. It may, however, be worth while to consider whether the metabolic differences are not primary, and whether the primitive gonad may not develop into an ovary in one case and a testis in the other in consequence of a fundamental difference existing in all the cells. In vertebrates the secondary sexual differences are largely dependent on the presence of a functional ovary or testis, but in insects this is not the case, and if Steche's observations are substantiated they indicate that all the cells of the body are different in the two sexes. Further, the existence of occasional gynandromorphs in vertebrates, in which one side of the body has male characters, the other female, while the gonad contains both male and female elements, shows that there must be some difference in the tissues of the two sides of the body on which the secretion of the gonad can act.[1] If, then, there is any truth in the conception here outlined, the presence or absence of a chromosome does not affect the primary sexual organs directly, but, perhaps by its presence in every cell, alters the whole metabolism in such a way that the organism is caused to become of one sex rather than of the other, in consequence of its type of metabolism. There is no necessary reason, however, for supposing that other causes might not alter the metabolism in the same way. Geoffrey Smith has, in fact,

[1] This was pointed out by C. J. Bond at the British Association, 1913.

indicated the means by which it is affected in sacculinised crabs, and it is conceivable that other causes, such as "staleness" of the eggs before fertilisation, might from the beginning change the metabolism (perhaps by destroying the activity of a "sex-chromosome," as suggested by Pearl and Parshley (**50**)), in such a way as to cause an originally female-producing egg to develop into a male.[1]

The general conclusion must be that although the observations connecting a particular chromosome with the determination of one sex are in many cases indisputable, there is no evidence to show how this chromosome acts ; and that, since the sex of the offspring is in some cases modifiable by environment, it is probable that the presence of the chromosome is associated with a particular kind of cell-metabolism, of which sex is to be regarded rather as a visible expression than as a cause.

SUMMARY.

In the first section a summary is given of the main lines of argument leading to the conclusion that "Mendelian characters are determined by chromosomes." Some indication is given of the restrictions which must be placed on the meaning of this phrase in respect of the part played by the cytoplasm in heredity. It is concluded that the arguments in its favour, though very strong indirectly, are not supported by sufficient direct evidence to be regarded by themselves as indisputable.

In the second section the chief classes of facts are reviewed which suggest a relation between chromosomes and sex-determination, and a preliminary account is given of a new case of an unpaired "sex-chromosome" in the female, in a strain of the moth Abraxas. It is concluded that the arguments for a relation between chromosomes and sex are much

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[1] The production of workers from female-producing eggs of the Hymenoptera is possibly a comparable instance. A change of metabolism induces fundamental differences in structure between individuals whose inherited constitution is identical, and these differences are of essentially the same nature as those which distinguish the two sexes.

stronger than those connecting chromosomes with Mendelian factors.

In the third section the facts of sex-limited inheritance are discussed; these are regarded as strongly reinforcing the arguments of the two preceding sections.

Lastly, certain difficulties are considered, and it is concluded that sex cannot be determined directly by the presence or absence of a factor which merely determines whether an ovary or a testis shall develop, but that the determining factor causes a certain type of metabolism, which in turn leads to the production of one sex or the other. If such a metabolism is induced by other causes, an individual of one sex may probably arise from gametes which, in the absence of disturbing causes, would have given rise to the other sex.

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A Cytological Study of Œnothera mut. lata and Œ. mut. semilata in Relation to Mutation.

By

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With Plates 35, 36, and 37, and 4 Text-figures.

Introduction.

THE special interest which attaches to the genus Œnothera at the present time arises from the description by de Vries of the mutation phenomena in these forms. The observations of de Vries have subsequently been confirmed by various investigators, but there has been much difference of opinion concerning the nature, causes and significance of the mutations themselves as they occur in Œ. Lamarckiana and Œ. biennis.

The two main views which have been expressed regarding the origin of the mutants have been (1) that they arise through a germinal change or a mutation in the de Vriesian sense, (2) that they are all merely re-combinations of Mendelian characters already present in the parent form.

One of us (Gates, 1909a) was able to show that in the mutant, Œ. gigas, the cells are of gigantic size, and that this explains most of its peculiarities. The giantism of the cells is associated with a doubling in the chromosome content of their nuclei (Lutz, 1907, Gates, 1908b). Œ. mut. gigas is therefore a tetraploid species, having 28 instead of 14 chromosomes, and in this respect it resembles many wild species in Nature (see Gates, 1913b).

It has thus been shown that tetraploidy is a condition which may, and probably always does, arise suddenly in Nature; and the appearance of tetraploidy as it occurs in many plants and animals is thus shown to be a progressive evolutionary process of considerable interest.

The present paper is concerned with another change in chromosome number obviously of a different character, namely, the occurrence of 15 chromosomes in Œ. mut. lata and its relatives. Miss Lutz (1912) has recently examined the root-tips of twenty-eight lata plants and reports in every case 15 chromosomes, and one of us (Gates, 1912b) in a study of the megaspore mother-cells and nucellus of a lata plant, found the number of chromosomes also to be 15. The investigations here described were begun to determine whether this number was constantly associated with the lata characters, and what numbers were to be found in related forms such as Œ. mut. semilata; also what chromosome numbers were present in mutants with lata-like foliage which occurred in other species, e. g. Œ. biennis L., and certain hybrid races (Œ. mut. rubricalyx × Œ. grandiflora Solander), which will be referred to later.

For this purpose a cytological examination of many plants from various sources has been made, the material for study having been collected in all cases from plants in our own pedigree cultures. These plants were grown at the John Innes Horticultural Institution in 1912, and we are indebted to Prof. Wm. Bateson, F.R.S., for the facilities afforded.

METHODS.

The flower-buds were all collected between July 31st and August 20th, 1912, buds of all ages being fixed. The fixing fluids used were 1 per cent. chrom-acetic acid, weak Flemming and Hermann's solution. The chrom-acetic fixation was found to be most suitable for this material and this type of investigation in which the number and morphology of the chromosomes was the main concern. The microtome sections were

cut chiefly at a thickness of 10 μ and stained with Heiden-
hain's iron-alum-hæmatoxylin without a plasma stain.

The chromosome counts were made as far as possible in
pollen mother-cells undergoing the heterotypic and homo-
typic mitoses, checked by a large number of counts from
somatic prophase and metaphase stages in young petals or
young ovary tissue.

MATERIAL.

In the following table is given the pedigree number,
history, and number of chromosomes in each plant examined.
As mentioned above, these plants were all grown in 1912 at
Merton. Under the pedigree number of each plant is given
(1) the number of the culture, (2) in Roman numerals, the
number of the row in which the plant occurred, and (3) the
number of the plant in the row. Thus, 228 . IV . 17 means
plant number 17 in row IV of culture 228. Hence it is
possible to refer to any individual plant by its pedigree
number.

From the table it will be seen that every plant having
lata or semilata foliage characters has also 15 chromo-
somes, and that this is quite irrespective of the source
from which the plants came. In all, 21 plants having lata
or semilata foliage were examined, and they originated
in diverse races of Œnothera from Sweden, Hungary,
Amsterdam, Madrid, and Birkenhead (England), both in pure
races and in hybrid cultures. On the other hand, it is known
from independent work of Geerts (1907), Gates (1907b), Lutz
(1907) and Davis (1911), that the number of chromosomes in
Œ. Lamarckiana is constantly 14 ; also that it is 14 in the
mutants rubrinervis (Gates, 1908a), rubricalyx (here
communicated), nanella (Gates, 1908b, Lutz, 1908), and in
Œ. brevistylis (unreported) and Œ. lævifolia (Gates,
1909b). The number 14 has also been shown to be present
in the normal species Œ. biennis L. (Gates, 1909b, and
Davis, 1910) and in the other species Œ. grandiflora

TABLE I.

Plant.	Pedigree number.	Number of chromosomes.	History of culture.
Œ. mut. semilata	228 . IV . 17	15	"Komb. 6" × Œ. Lamarckiana, received from N. Heribert-Nilsson, from Sweden. For a further account of these and other cultures see below.
Œ. mut. lata	228 . IV . 20	15	
Œ. lata to semilata	226 . II . 18	15	A race of Œ. Lamarckiana received from the Kolosvar Botanic Gardens, Hungary.
Œ. mut. lata	226 . III . 1	15	
Œ. mut. lata	229 . II . 17	15	"Komb. 1" (lata-like) from N. Heribert-Nilsson, Sweden (see Heribert-Nilsson, 1912), described below.
Œ. mut. lata	229 . II . 13	15	
Œ. mut. lata	229 . I . 24	15	
Œ. mut. lata	229 . I . 11	15	
Œ. mut. lata	229 . I . 4	15	
Œ. mut. semilata	229 . I . 6	15	
New lata-like mutant	229 . I . 10	15	
Œ. semilata to lata	229 . I . 12	15	
Œ. semilata to lata	229 . I . 17	15	
Œ. mut. lata	179 . I . 1	15	From lata × lata of de Vries's cultures.
Œ. mut. lata	179 . I . 2	15	
Œ. mut. lata	142 . I . 1	15	From Œ. mut. semilata, self-pollinated.
Œ. Lamarckiana	142 . I . 2	14	
Œ. mut. lata	142 . I . 3	15	
Œ. biennis mut. lata	213 . VII . 5	15	From a race of Œ. biennis from the Madrid Botanical Garden.
Œ. mut. semilata ("English lata")	114 . I . 1	15	From Birkenhead, England, 1906.
Œ. near "English lata"	114 . I . 4	15	
Œ. mut. rubricalyx	168 . VII . 1	14	A pure homozygous culture of this mutant.
Œ. mut. lata rubricalyx	60 . I . 20	15	In F₂ of Œ. mut. rubricalyx × Œ. grandiflora. (See below.)

Solander (Davis, 1909), as well as in Œ. longiflora (Beer, 1906), Œ. muricata, Œ. cruciata and Œ. Millersi (Stomps, 1912). It will, therefore, be obvious from these facts, together with the description of the cultures above enumerated, that plants having the foliage characters of lata or semilata and 15 chromosomes arise sporadically from races having 14 chromosomes.

Œ. MUT. LATA AND SEMILATA.

Before proceeding with the description of the plants in these cultures, it will be necessary to point out the chief peculiarities of lata and semilata. These forms were first described by de Vries (see 1909, vol. i, p. 415), in whose cultures lata appeared as a mutant from Œ. Lamarckiana and several of its derivatives to the number of 493 plants in a total of 130,000 seedlings, or with a frequency of about 0·4 per cent. The most reliable percentages obtained in families of 8000, 10,000 and 14,000 plants respectively were 0·5 per cent., 1·7 per cent. and 1·8 per cent., while in one small culture of 164 plants the number of latas ran up to 5 per cent. These fluctuating frequencies are doubtless an indication of environmental influence.

In de Vries's cultures, semilata only appeared in the off-spring of lata × Lamarckiana. Œ. lata was classed by him as an inconstant species, but semilata was incorrectly classed as constant. They are both obviously inconstant, however, and the presence of the odd chromosome shows why this must be so.

Text-fig. 1 shows a typical rosette of lata, as it occurs in cultures from de Vries's race of Lamarckiana. The leaves are broad, with usually very obtuse, rounded points, and very deeply crinkled. Lata also has a peculiarly hand-shaped stigma, the buds are stout and barrel-shaped, usually more or less symmetrical, with the petals crumpled inside the bud. The anthers are almost wholly sterile, and in typical plants there is a complete absence of red pigment from all

TEXT-FIG. 1.

Typical rosette of Œnothera lata of de Vries.

TEXT-FIG. 2.
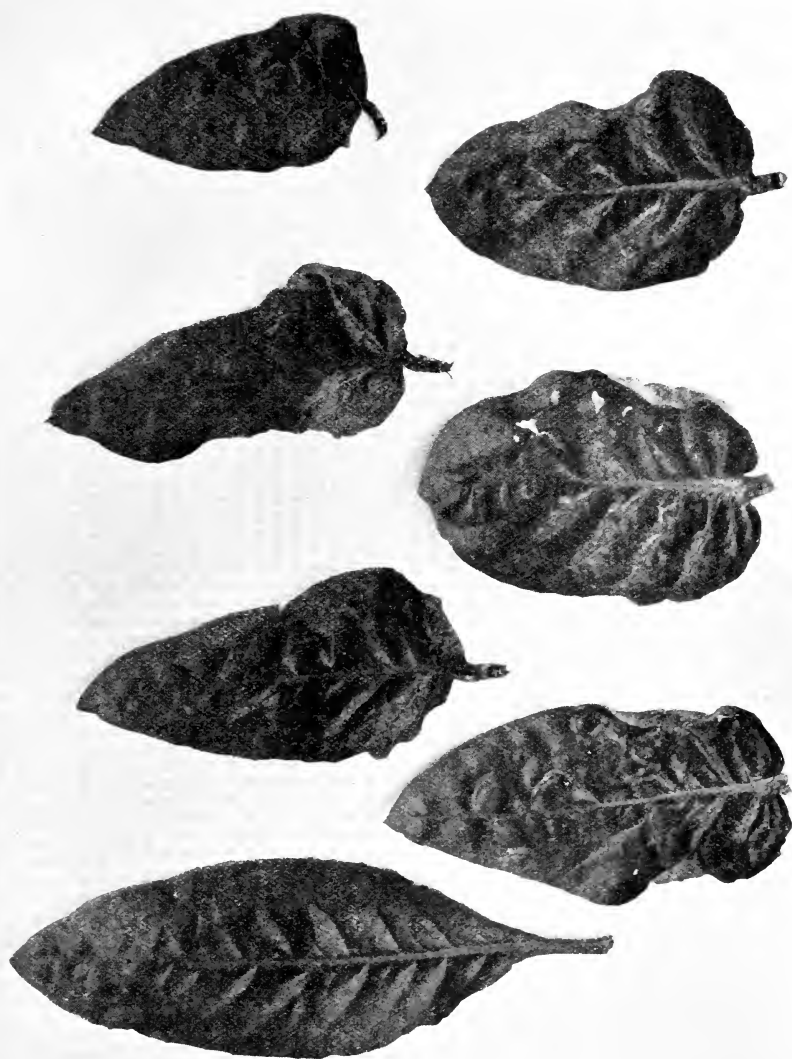
Upper row.—Stem-leaves of Œnothera semilata of de Vries.
Lower row.—Stem-leaves of Œnothera lata of de Vries.

parts (see Gates, 1913a, pl. iii, figs. 42, 43 left). The plants
are also peculiar in habit, about half as high as Lamarckiana,
having weak, more or less decumbent stems and sprawling
branches.

The typical semilata stands about midway between lata
and Lamarckiana. Its leaves are more pointed and rather
less crinkled than those of lata. The stem is erect and taller
than lata, though shorter than Lamarckiana, the buds are
less stout and more squarish than lata, and it produces a
considerable quantity of pollen (see Gates, 1913a, figs. 43
right and 44).

Text-fig. 2 contrasts the stem leaves of lata (lower row)
and semilata (upper row) as they appear in mutants derived
from cultures of de Vries's race of Lamarckiana. These
two types from this source are remarkably uniform whenever
they appear, but in some of the cultures from other sources
they are much more variable, so that it becomes difficult or
impossible to draw a line between lata and semilata.

Description of Cultures.

We may now describe the main features of the cultures in
the order in which they are enumerated in Table I. Culture
228 was derived from seeds sent by the kindness of N.
Heribert-Nilsson from Sweden. They were descended from
his pedigree cultures of a race of Œ. Lamarckiana, found
by him in a garden in southern Sweden, which differs in
various features from the Lamarckiana of de Vries's
experiments. " Komb. 6 " of Heribert-Nilsson (see 1912,
p. 128) most resembles Œ. mut. rubrinervis, from which
it differs, however, in certain features. Our culture of
" Komb. 6 × Lamarckiana " contained 120 plants, 110 of
which belonged to the rubrinervis type, differing from the
de Vriesian mutant chiefly in having, for the most part,
smooth leaves. The buds bore an extreme amount of red
pigmentation for rubrinervis, i. e. the red colour pattern 7
on the sepals, and reddish streaks on the hypanthium (see

Gates, 1911b, pl. vi, for the colour variations of rubrinervis buds). Four other plants differed from these only in having the more typical rubrinervis colour pattern (5). Of the remaining plants, three bore buds somewhat smooth and rounded, resembling those of Œ. grandiflora; one (IV, 17) was a semilata whose leaves had pinkish midribs; one (IV, 20) was lata with pinkish midribs, and one was a very aberrant, bushy type, having small, elliptical, finely serrate leaves with short petioles and rounded buds with no long hairs. The rough brown stem and small leaves gave this peculiar plant an almost ericaceous aspect. It was completely sterile in both anthers and ovaries.

Of this culture, then, 117 plants resembled rubrinervis closely in foliage; one belonged to semilata, one to lata, and one to a very aberrant and sterile type. Unfortunately, no cytological material was collected from the latter plant.

Culture No. 226 was derived from seeds received from the Kolosvar Botanic Garden under the name Œ. biennis L. grandiflora Hort. It contained 116 plants, which were mostly Lamarckiana and rubrinervis, with rather variable foliage, but included also two nanellas, one (II . 18) lata to semilata, having a little pollen and pink midribs, one (III . 1) typical lata.

Culture No. 229 came from open-pollinated seeds of Heribert-Nilsson's lata-like "Komb. 1." It contained 79 plants, most of which belonged to a type whose buds resembled those of grandiflora, the leaves being nearly smooth, cordate. But there were also one nanella, one semilata (I . 6), two lata to semilata (I . 12 and II . 17), five lata (I . 4, I . 11, I . 17, I . 24 and II . 13), and one new type (I . 10) like a small, weak lata, having broad-pointed, crinkled leaves nearly cordate at base, and rather small flowers. Hence the foliage and habit of the latter plant alone resembled lata, and, as might have been anticipated, the plant, like the eight lata and semilata plants above mentioned, had 15 chromosomes.

Cult. No. 179 contained only two plants, both typical

lata, derived from the pollination of one lata plant with
a small quantity of pollen produced by another.

The history of cult. No. 142 is as follows: A packet of
lata seeds kindly sent by Professor de Vries yielded in 1909
seventy-five plants, all of which were Lamarckiana except
eight. Of the latter, four were lata, three semilata, and
one aberrant. One of the semilatas self-pollinated produced
in 1911 forty plants, of which three came into bloom and
were semilata. The others remained rosettes and as such
could not all be classified, though twenty-two of them were
Lamarckiana and ten formed a variable semilata series.
One of the three semilatas which bloomed was again selfed,
and yielded cult. 142 in 1912. The latter consisted of
only three plants, two of which were typical lata with
15 chromosomes (see table), and one Lamarckiana with
14 chromosomes. The latter, however, differed from typical
Lamarckiana in having nearly smooth leaves, some of
which were cordate in shape.

Thus semilata gives Lamarckiana offspring, having
14 chromosomes, and latas with 15 chromosomes, as well as
semilatas. Lata when selfed gives Lata and Lamarck-
iana and other mutations as well, while lata × Lamarck-
iana yields lata, Lamarckiana and semilata, with
occasional others.

Cult. No. 114 was grown from seeds of Œnothera
obtained from Birkenhead, England, in 1906. The culture
contained six plants, of which two belonged to a type we call
" English lata," which closely resembles Œ. semilata.

One of the most interesting plants in this series of lata
and semilata forms is found in cult. 213. This culture,
which has been briefly described elsewhere by one of us
(Gates, 1912a), was a race of Œ. biennis, derived from the
Madrid Botanic Garden in 1909 : 122 plants were brought
to maturity in 1912, of which 23 belonged to a type having
Lamarckiana-like foliage, one was more finely crinkled,
91 resembled rubrinervis in having red midribs, 6 bore
narrow, furrowed leaves like lævifolia, and 1 had the

foliage of lata. All these plants had the small flowers
of Œ. biennis. The Œ. biennis lata mutant again had
15 chromosomes, though normal biennis contains 14, the
fundamental number in the genus Œnothera. The lata
mutant is shown in Text-fig. 3 (p. 534), while Text-fig. 4
(p. 535) shows the rubrinervis type for comparison.

Another lata type of equal interest to the last appeared
in the F_2 of Œ. mut. rubricalyx × Œ. grandiflora.
This series of hybrids is described elsewhere. Two lata
plants occurred in one F_2 culture numbering 56 plants, but
only one of them (60 . I . 20) came to maturity. They were
essentially plants with lata foliage and habit, together with
the remarkable red pigmentation of rubricalyx. The
plant which matured produced flowers with an abundance of
pollen, and developed long stout capsules, unlike the typical
lata, which is almost completely male-sterile and whose
capsules are short and contain few seeds. In some unknown
way this sterility had been largely overcome. It is not clear
whether the presence of great quantities of anthocyanin,
which was obviously inherited from the rubricalyx grand-
parent, exerted any effect. This plant had again 15
chromosomes, though in both mut. rubricalyx and
Œ. grandiflora the number is 14. The behaviour of
the chromosomes in this plant will be described in detail.

From these facts we are justified in concluding that plants
having the foliage and habit of lata or semilata, even
when these are combined with other characters such as small
flowers or red pigmentation inherited from their ancestors,
have constantly 15 chromosomes.

CYTOLOGICAL DESCRIPTION.

Somatic mitoses.

In the previous section we found that a total of 21
individuals which in foliage and habit belonged in the lata-
semilata series of forms, all without exception contained

TEXT-FIG. 3.

Œnothera biennis mut. lata.

TEXT-FIG. 4.

Œnothera biennis—"rubrinervis type."

15 chromosomes. These individuals belonged to eight
distinct cultures from various diverse sources, numbering in
all 504 plants. They, moreover, were all derived, immediately
or more remotely, from races having 14 chromosomes.

Certain cytological features of these 21 individuals may
now be considered, beginning with the somatic divisions.

In cult. 228, although seventeen buds of plant No. IV. 17
(semilata) were microtomed, no reduction divisions were
found; but over thirty counts of somatic mitoses showed the
number of chromosomes to be constantly fifteen. Figs. 1–7
(Pl. 35) are drawn from somatic divisions in this individual,
showing two prophase and five metaphase stages. In some
cases the chromosomes are evidently in pairs and the odd or
extra chromosome can be identified without much difficulty
(figs. 5–7), but in the majority of cases the pairing is not so
marked (figs. 3, 4). There is, therefore, no constancy in the
degree of pairing in the various metaphase figures of an
individual, though the odd chromosome is often easily dis-
tinguished.

There is considerable variation also in the size, shape and
thickness of the chromosomes, though their number is
constant. Fig. 5 shows a case where one chromosome is
considerably smaller than the remaining fourteen, a fairly
common variation in the lata plants, shown also in biennis
lata (fig. 8). Occasionally, as in fig. 7, one chromosome has
a median constriction giving it a dumb-bell appearance, and
not infrequently a constriction appears near one end of a
chromosome, as in fig. 9, more or less completely pinching off
a small portion.

In the lata plant No. 228. IV. 20 over twenty exact
somatic counts were made, the number of chromosomes being
15 in each case. Fig. 10 shows a clear pairing of certain
chromosomes in a nucleus of this individual; and in fig. 11
one of the chromosomes is exceptionally small, and the pairs
are very well marked.

In making chromosome counts the utmost care was
exercised, and only nuclei were selected in which the number

of chromosomes present could be determined with the utmost exactitude. Moreover, in each individual the chromosomes in a number of nuclei were counted independently by us both, the same conclusions being reached in each case. The constancy in chromosome number is such that a few perfectly accurate counts are amply sufficient to determine the number present in any individual, though a larger number of counts were made in nearly every case.

In plant No. 226 . II . 18, which was intermediate between typical lata and semilata, fourteen counts made in the petals and ovary gave 15 chromosomes in every case. In addition, six counts of the heterotypic metaphase each gave 15 chromosomes, and numerous later stages of meiosis showed the 7 + 8 distribution of the chromosomes in the reduction division, which will be discussed later. No. III . 1 of this culture, a typical lata plant, also showed 15 chromosomes, both in its somatic and meiotic divisions.

In cult. 229, the nine plants having lata or semilata characters all possessed 15 chromosomes. In one of these plants (No. I. 4) several cases were observed in which the somatic chromosomes at metaphase were more or less completely divided transversely into two (see figs. 12 and 13). A light area in which not even linin could be detected was found usually in the middle, but sometimes nearer one end of each chromosome. A similar condition was observed by Fraser and Snell (1911) in Vicia faba, but only in one or two of the chromosomes, whereas in the present case nearly all the chromosomes of a group are thus divided. Similarly, Agar (1912) has found in Lepidosiren that the chromosomes frequently undergo a transverse segmentation which includes the chromatin, but not the linin portion of the chromosome. This segmentation does not always occur in the middle, but sometimes nearer one end, and moreover, the point at which constriction or segmentation takes place is found by him to be constant for each chromosome. Numerous other cases of transverse segmentation are to be found in the literature.

In a semilata plant (I . 6) was found the condition shown

in fig. 14, in which the chromosomes in metaphase have begun their fission at one end. There are here sixteen or seventeen chromosomes, the extra ones being evidently due to a precocious completion of the fission by certain chromosomes. This is an occasional source of apparent variation in the chromosome number. One other observed case of 16 chromosomes in the somatic metaphase is doubtless to be accounted for in the same way, and was not a real variation. This is further shown by the fact that counts in the prophase of mitosis have never been found to show any variation in number.

In another lata plant (142 . I . 3) a similar case of the precocious fission of certain chromosomes was found (fig. 15). In this group the chromosomes varied much in size and were strongly paired, both members of one pair (on the right) having undergone a precocious split, while a third chromosome is beginning the fission from both ends, the remaining chromosomes showing no indication of a split.

We regard these cases as a demonstration that certain chromosomes of a group sometimes divide prematurely, and there can be little doubt that this has led in some cases to an assumption of variation in the number of chromosomes where such variation does not actually exist. It seems probable that such premature splitting of chromosomes will be found to occur most frequently in hybrids and mutating forms.

Meiotic Divisions.

The behaviour of the 15 chromosomes during meiosis was especially studied in several plants. The normal features of meiosis in various Œnotheras are already well-known. Hence in the present paper we shall confine ourselves chiefly to pointing out various peculiar features in connection with the behaviour of the extra chromosome and the phenomena of chromosome degeneration.

We may first point out that in the heterotypic mitosis in

the lata and semilata forms, the 15 chromosomes are
usually distributed so that 7 go to one daughter-nucleus and
8 to the other.   In rare cases the distribution is 9 and 6.
Only four such cases were observed, in one of which one
chromosome also divided, making the distribution $9\frac{1}{2} + 5\frac{1}{2}$.
More frequently the extra chromosome is left behind, where
it may be seen fragmenting and degenerating in the cyto-
plasm.   In such cases the four pollen grains formed from a
mother-cell will each contain 7 chromosomes.   Hence while
theoretically equal numbers of germ-cells having 7 and 8
chromosomes would be anticipated, actually the number
having 8 is a much smaller proportion.

A still more interesting fact with regard to the cytological
behaviour in these plants is that one chromosome, which is
probably the extra chromosome, not infrequently divides
longitudinally in the heterotypic mitosis, its two halves passing
to the daughter-nuclei.   That is, unlike the other chromo-
somes, this one divides in the first instead of the second
reduction division.

The later history of these half-chromosomes is of much
interest.   We have observed no case in which they pass
undivided to one pole of the homotypic spindle.   In normal
homotypic anaphases we have invariably found the chromo-
somes all dividing regularly and their halves passing to the
poles.   But we have observed homotypic metaphase groups
(cf. fig. 24) containing each an apparently normal half-
chromosome, and in such cases we believe that the half-
chromosome will either degenerate or, as a rare occurrence,
pass undivided into one of the tetrad nuclei, thus producing a
pollen grain with 8 chromosomes.   There is no reason for
supposing that such pollen grains would differ from those
formed by the regular division of 8 whole chromosomes on the
homotypic spindle.   In any case, it is highly improbable that a
chromosome which has undergone fission in the heterotypic
mitosis will divide again in the homotypic, unless merely as a
fragmentation accompanied by degeneration ; and hence for
such chromosomes the second will be the reducing division.

This division of what is presumably the extra chromosome on the heterotypic spindle is often not a regular one, but consists in an irregular pulling apart of the chromosome into two, or sometimes a fragmentation into three parts. Moreover, this behaviour is not always confined to a single chromosome, for occasionally two chromosomes may be seen to divide on the same heterotypic spindle, and this condition has even been found in a Lamarckiana plant derived from semilata. Hence, assuming that this peculiarity of dividing in the first division is characteristic of the extra chromosome, it cannot be confined entirely to that chromosome, but sometimes extends to one of the others. This behaviour has never been found, however, in pure races of Lamarckiana or its mutants having 14 chromosomes, so that it appears to be connected with peculiarities and disturbances arising originally from the presence of the extra chromosome.

Œ. MUT. LATA RUBRICALYX.

Returning now to the detailed description, we shall first describe the phenomena of meiosis as regards the history and fate of the chromosomes in lata rubricalyx. The history of the origin of this 15-chromosome plant from hybrids of rubricalyx and grandiflora having 14 chromosomes has been referred to above (see p. 533), and will be discussed elsewhere (Gates, 1914). Fig. 16 is a profile view of the heterotypic spindle in a pollen mother-cell of pure rubricalyx, clearly showing the 14 chromosomes. The loosely scattered and more or less unpaired condition of the chromosomes at this time is characteristic of Œnothera. Fig. 17 is a polar view of the same stage, showing the same chromosome number, the chromosomes occurring in several foci.

Figs. 18 to 31 are studies of various stages in the meiotic divisions of lata rubricalyx. They disclose much variation in the behaviour of the chromosomes.

Fig. 18 shows diakinesis in a pollen mother-cell. The 15 chromosomes are, as usual, scattered in their arrangement,

and two nucleoli are also present. Fig. 19 shows the hetero-
typic spindle in a pollen mother-cell in metaphase, all the 15
chromosomes being represented. In this cell the cytoplasm
is vacuolate and the chromosomes small, so that the cell is pro-
bably beginning to degenerate. In figs. 20, 21 are shown polar
views of two mother-cells in the same stage. Each contains
15 chromosomes, but they are much smaller than the normal
size, and the cells show signs of approaching degeneration.

From these and following figures it will be seen that the
chromosomes in lata rubricalyx vary enormously in size in
different cells, though relatively uniform in size in the same
cell, the variation in size depending apparently upon the con-
ditions of nutrition and prospects for development of the cell.
Fig. 22 shows a portion of a normal mother-cell of this plant.
This is a late anaphase of the heterotypic division and the
spindle has been cut, so that only 11 chromosomes can be
seen. But these are full size, and each shows the fission
which occurs usually as the chromosomes are passing towards
the poles of the spindle. The chromosome-halves thus formed
remain paired in this way during interkinesis and then finally
pass to opposite poles of the homotypic spindle. Fig. 23
shows one of the two daughter-nuclei of a pollen mother-cell
in the interkinesis stage after the heterotypic mitosis. In
this particular case the nucleus contains 7 chromosomes all
showing their bivalent character, and a small nucleolus. In
addition, an eighth chromosome has been left just outside the
nucleus. This is the extra or fifteenth chromosome which has
just failed to reach the daughter-nucleus, as not infrequently
happens. It will later degenerate, and such a mother-cell
would therefore produce four pollen grains, each containing
7 chromosomes.

Figs. 24 and 25 are polar views of the homotypic metaphase
in lata rubricalyx. In fig. 24 each group contains 7
whole chromosomes which are more or less clearly split, and a
half-chromosome. These two halves, which lie facing each
other, are obviously derived from the fission of the extra
chromosome in the heterotypic metaphase. They are about

the same size as the other chromosome-halves.   Certain other
cases which show this even more conclusively will be
described later.   In fig. 25 the cell, nuclei and chromosomes
are all distinctly smaller than in fig. 24, and the chromosomes
show very little evidence of a split.   The left-hand group
contains $7\frac{1}{2}$ chromosomes, while the right-hand group con-
sists of 6 whole chromosomes, a half-chromosome and a
small fragment.   There is in addition a chromosome in the
cytoplasm near this group, which has been left out of the
nucleus in interkinesis.   This makes the full quota of 15
chromosomes.   The origin of the fragment is obscure, but
the figure shows that even fragments of chromosomes may be
distributed occasionally to the daughter-nuclei.   Unless
afterwards extruded from the pollen nuclei, such fragments
would no doubt affect the later development of the indi-
vidual; they might remain independent or become attached
to or fused with one of the other chromosomes.   These
unequal divisions of chromosomes furnish an obvious source
of variability within certain limits—such variability as is
found in the lata-semilata series of forms.

Fig. 26 is a profile view of a homotypic spindle in very
early anaphase when the chromosomes are just separating.
In figs. 27–31 are represented later stages of the homotypic
anaphase, showing the distribution of chromosomes to the
pollen tetrads.   Fig. 27 a and b shows the two homotypic
spindles in a mother-cell, degenerating chromosomes being left
behind in the median region of both spindles.   In b there are
8 chromosomes in a group near each pole, and in addition
two fragments which stain less deeply and are evidently
degenerating without being drawn to the pole.   In a there
are only 6 chromosomes at either pole, and again two frag-
ments remain behind to degenerate.   It is very probable that
these fragments are derived in each case from a half of one
chromosome which underwent fission on the heterotypic
spindle.   There is also much variation in the size of the
chromosomes in this cell, those in b being on the average
somewhat larger than those in a.

In fig. 28 one homotypic spindle of another mother-cell is represented, showing 8 chromosomes in each anaphase group. In the mother-cell represented by fig. 29, all the chromosomes in the cell could be observed, showing very clearly 7 chromosomes in each of the anaphase groups on the upper spindle and 8 in each group on the lower spindle. This is the condition which should normally occur in all cases, giving two pollen grains with 8 and two with 7 chromosomes. In fig. 30 *a* and *b* the full number of chromosomes is present, though they vary greatly in size and some are degenerating. The left-hand spindle contains 6 chromosomes at one pole, 4 at the other, and 4 more degenerating in the median region of the spindle, showing that this spindle received 7 of the heterotypic chromosomes. The other homotypic spindle, which was cut at one end, contains 8 chromosomes at or near either pole.

The mother-cell represented in fig. 31 *a* and *b* is in a late stage of the homotypic anaphase, the spindle on the left showing two clear groups of 8 chromosomes. On the right, one group of 7 is clearly countable; the other group has been cut. This group also contains 7 chromosomes, 5 together and 2 displaced by the knife. One of the latter (marked *c*) has also been cut, the greater portion of it lying in section *a*, while a small part is in section *b*.

It should be pointed out that only normal mitotic figures without degenerating chromosomes were used for making the chromosome counts, and cases such as those just described, where difference of interpretation regarding the valency of particular chromosomes was occasionally possible, were not used at all as evidence in determining the chromosome-number for the plant.

In addition to the irregularities and chromosome degenerations already described, lata rubricalyx shows various conditions of degeneration of the pollen mother-cells themselves. Sometimes the whole anther fails to undergo proper development. In these cases (figs. 32, 33, 34) the tapetum wholly fails to differentiate and, probably as a result, the

pollen mother-cells contain very little cytoplasm.   Often the
cell-walls between the mother-cells of an anther are very
imperfectly formed or almost wholly lacking, so that their
protoplasm forms a continuous mass from one end of the
anther to the other (fig. 32).   The nuclei in these cases have
usually disappeared.   In such anthers the degeneration must
have begun in the archesporial tissue.   In other cases
(figs. 33, 34) the cell walls between mother-cells are
nearly complete, but nuclear material passes through
openings in the walls into the adjacent mother-cells.   This
may happen when the nuclear contents are fused into a
single mass (fig. 33), or when they are in discrete chromo-
somes (fig. 34).   In the latter case the chromosomes are
smaller than normal and frequently scattered in the cyto-
plasm.   A condition somewhat similar to that shown in
fig. 33 was described by Gates (1911a, fig. 9) in Œ. mut.
gigas.   In the latter case, however, the tapetum was
developed, though the mother-cells afterwards remained in
contact with it.   The nuclei were in synapsis, and showed no
signs of abnormality except in the extrusion of chromatin
into adjoining cells.   Similar conditions were described by
Miss Digby (1909) in Galtonia.

Fig. 35 represents a pollen mother-cell of Œ. lata rubri-
calyx in another condition of degeneration.   The cytoplasm
becomes highly vacuolate and scanty, while the nucleus
disappears, leaving only pale-staining scattered chromatin
remnants.   This condition is a fairly common one.

In addition to these peculiarities, cases of extra nuclei in
the pollen tetrads are common.   The extra nuclei are, of
course, formed by chromosomes left behind in the cyto-
plasm, as was shown in Œ. mut. rubrinervis (Gates,
1908a), Œ. mut. gigas (Gates, 1911a), and other forms,
where they occur, though less commonly.   It is not necessary
to figure them again here.   Such cells probably all degene-
rate, except perhaps in rare instances.

Œ. Biennis mut. Lata.

The study of the pollen development in biennis lata showed conditions similar to those in lata rubricalyx, but the latter plant produced a good amount of viable pollen, while the former was quite sterile. The reasons for these differences in sterility are not apparent.

Fig. 36 is a polar view of the heterotypic spindle in biennis lata, showing the 15 chromosomes. Figs. 38 and 39 represent the usual condition in the homotypic metaphase, showing the 7–8 distribution of chromosomes. In fig. 38 the spindles are at right-angles to each other, and all the chromosomes can be clearly counted. In fig. 39 the groups are seen in somewhat oblique view, so that a number of the chromosomes show the longitudinal split. Fig. 40 portrays an exceptional case. The two metaphase groups on the homotypic spindles contain respectively $6\frac{1}{2}$ and $8\frac{1}{2}$ chromosomes. Hence not only did one chromosome, probably the extra, split in the heterotypic division, but one other chromosome of the seven pairs also passed to the wrong pole of the spindle. This case is very clear, and admits of no other interpretation.

Fig. 41 a and b represents a case of a 9–6 heterotypic distribution of the chromosomes in biennis lata. The two homotypic spindles were at right-angles in the cell. Another similar group of 9 chromosomes was found in this plant, a third in a typical lata (No. 179 . I . 2), and in the lata-like plant, 229 . I . 10, one case of $9\frac{1}{2}+5\frac{1}{2}$ chromosomes was found.

Irregularities of this kind are occasional occurrences in all the 14-chromosome forms, and result in an 8–6 distribution of the chromosomes. There can be no doubt that to such irregularities are due the origin of all the lata and semilata series of mutants. These irregularities have been observed by one of us in Œ. mut. rubrinervis 1903a), lata × Lamarckiana (1910), and Œ. biennis (Gates), and by Davis in Œ. Lamarckiana (1911), and

Œ. biennis (1910). The corresponding 15–13 distribution was found in Œ. mut. gigas (Gates, 1911a) and a 9–11 distribution in a 20-chromosome plant, having lata as the mother (Gates, 1910).

In fig. 37 a somewhat different condition from any observed in lata rubricalyx was discovered, namely, a tendency for the chromosomes to leave portions of their viscous substance trailing behind as they pass towards the poles. This condition was not infrequently observed in biennis lata. It apparently denotes a pathological condition of the chromosomes concerned, and is probably followed by their degeneration.

Geerts (1911) has described the fragmentation and degeneration of certain chromosomes in the homotypic division in the pollen mother-cells of gigas × Lamarckiana, and in the first meiotic division of the megaspores of lata × gigas. He also found that during interkinesis in the pollen mother-cells of the last cross, some of the chromosomes (which would probably afterwards degenerate) failed to undergo the usual split. His figures also indicate that certain of the chromosomes may have split on the heterotypic spindle as here described.

Lamarckiana-like Plant (142 . I . 2).

Figs. 42 to 45 show certain features of the meiotic chromosomes in the Lamarckiana-like plant (142 . I . 2), having 14 chromosomes, derived from self-pollination of semilata. The heterotypic spindle drawn in fig. 42 contains 14 chromosomes, two of which are leaving behind trails of chromatin similar to those observed in biennis lata (fig. 37). Fig. 43 shows the normal homotypic metaphase with 7 chromosomes in each group, while in fig. 44 an irregular distribution has taken place, the result of which is not quite clear. But there appear to be about 6 whole chromosomes and a fragment in one group; and the other group contains 8 bodies whose valency is not clear in every case, though there are obviously great differences in the size of the chromosomes. In this

unusual case the distribution of chromatin has apparently not
been in strict accord with the law of chromosome individu-
ality.   It is one of the rare exceptions which serves to
emphasise the almost universal character of the rule.

The homotypic anaphase represented in fig. 45 is similar to
that shown for lata rubricalyx in fig. 29, except that the
number of chromosomes in each of the four groups is 7.

THE LATA-LIKE MUTANT (No. 229 . I . 10).

In all the lata forms the chromosomes appear to be rather
more viscous than in other Œnotheras, and hence there is
often an exceptional amount of variation in shape.   Figs. 46–
51 are taken from the lata-like mutant in a culture from
Sweden, already described (p. 531).   Fig. 46 is a heterotypic
metaphase showing 15 chromosomes.   Strictly speaking, in
Œnothera there is no heterotypic metaphase, for, with rare
exceptions, the chromosomes are at no time arranged in
regular paired alignment on the heterotypic spindle.   But
nevertheless there must be balanced forces controlling the
movements of the chromosome pairs at this time, since the
segregation of the chromosomes results, in nearly all cases,
in an equal distribution of the 14 bodies.   And there is
every reason to believe, from analogy with other cases, that
the chromosome pairs which separate on the heterotypic
spindle are the same as those which are so frequently obviously
paired in the metaphase of somatic mitoses.

In fig. 47 the heterotypic chromosomes are segregating so
that apparently 9 whole chromosomes will reach one pole of
the spindle and only 5 the other.   The 15th chromosome,
which is presumab'y the unpaired or extra chromosome, is
dividing, not by a regular longitudinal fission, but by pulling
apart in an irregular way, perhaps transversely, leaving a
trail of chromatin between the two halves.   Such a condition
was found a number of times in this plant.   Fig. 48, in which
the spindle appears to have been slightly cut, shows a similar
behaviour of the extra chromosome.   In fig. 49 one chromosome

is pulling apart in the same way, while another appears to be fragmenting into three pieces, and an additional chromatic fragment is found on the spindle.

Obviously, if pollen grain formation is completed in such cases and the pollen grains function, they will produce individuals having a different chromatin content in their nuclei. This, we believe, would lead to external variations in the offspring. Indeed, all the meiotic irregularities described in this paper may be looked upon as germinal variations, though the great majority of them will come to naught because the germ-cells, in which they occur, fail to reach maturity on account of their aberrant nature.

The two homotypic spindles in a pollen mother-cell of this plant are shown in prophase in fig. 50 a and b. They contain respectively 7 and 8 chromosomes, nearly all of which clearly show their bivalent character. They are about to be drawn into regular alignment on the equatorial plate. In this case the heterotypic segregation was obviously into 7 and 8 whole chromosomes. In fig. 51 one group of chromosomes in the homotypic metaphase is shown. There are present 8 chromosomes and one other which was obviously left out of the daughter-nucleus during interkinesis.

As will be seen from figs. 46–49, there is in this plant also great variation in the size of the chromosomes in different cells, and irregularities tend to be greater in the less nourished cells which have smaller chromosomes.

During synapsis and diakinesis in Œnothera (Gates,1908a), one, and sometimes two or more nucleoli are present in the pollen mother-cell nucleus. When the nuclear membrane breaks down in the heterotypic prophase, these nucleoli usually disappear immediately, leaving not a trace behind. But in certain circumstances they persist for a longer time, appearing on or close by the spindle. Fig. 52 shows such a case in lata, in which two nucleoli, in addition to the full quota of chromosomes, are found on the spindle. These nucleoli are distinguishable from the chromosomes by their perfectly spherical shape and by their lighter centre. They

gradually diminish in size and finally disappear. In an earlier paper by one of us (Gates, 1907a) they were considered problematical bodies and provisionally called heterochromosomes. Since their origin from nucleoli is now proved, this name may be discarded. Fig. 48 also shows a small nucleolus persisting on the heterotypic spindle.

Two other figures represent certain conditions in plant No. 226 . II . 18, having characters intermediate between lata and semilata. Fig. 53 shows a homotypic metaphase group with 7 chromosomes, the two halves, in one chromosome particularly, being rather widely separated. Fig. 54 shows a homotypic metaphase stage in polar view, in which the right-hand group clearly contains 6 whole chromosomes longitudinally split, and one half-chromosome. The left-hand group contains $7\frac{1}{2}$ chromosomes, and between the groups are fragments of the fifteenth chromosome, which is degenerating after being left behind on the heterotypic spindle.

The variety of behaviour thus exhibited by certain chromosomes in these plants include (1) the division of one chromosome (probably the extra one) in the heterotypic mitosis, this behaviour sometimes extending to a second, and occurring also in 14-chromosome plants descended from 15-chromosome individuals ; (2) the fragmentation and later degeneration of certain chromosomes on the heterotypic and homotypic spindles ; (3) the loss of chromatin from one or more chromosomes by its trailing behind on the heterotypic spindle ; (4) the leaving behind of certain chromosomes, to degenerate on the heterotypic spindle ; (5) the formation of extra nuclei by lagging chromosomes in the heterotypic and homotypic mitoses.

DISCUSSION.

From the facts above presented we may conclude, not only that the mutants lata and semilata constantly possess an extra chromosome, but that whenever, in any race, or species or hybrid of Œnothera, individuals occur having the general type of foliage characteristic of lata and semilata, such

individuals will have 15 chromosomes. The origin of such
mutants is clear. They originate through one of the
heterotypic chromosomes passing into the same daughter-
nucleus as its mate, instead of into the opposite nucleus.
This occurrence was first discovered by one of us (Gates,
1908a) in the mutant rubrinervis, and has since been
observed in several other mutants and species. As for-
merly pointed out, it is made possible by the weak attraction
between the homologous chromosomes during diakinesis
and the heterotypic mitosis. We may therefore predict that
in other genera showing similar cytological peculiarities,
sporadic mutants of a similar aberrant type will be found
to occur.

Whenever this irregular meiotic division occurs in a pollen
mother-cell, such a cell will, at least in many cases, give rise
to two lata-producing pollen grains in addition to two
having only 6 chromosomes. The latter apparently always
degenerate. Similarly, when such an irregularity occurs in
the megaspore meiosis, if the 8-chromosome megaspore
functions it will, after fertilisation by a 7-chromosome pollen
grain, give rise to a lata-like mutant. The frequency of the
occurrence of this irregular division in 14-chromosome plants
may determine the frequency with which lata-like mutants
will appear. Moreover, in lata or semilata when crossed
with their 14-chromosome parents or when self-pollinated, the
percentage in which the mutant reappears will depend upon
the relative number of their 8-chromosome and 7-chromo-
some germ-cells which function.

The frequency of the original unequal division in meiosis is
difficult to determine cytologically, but from the observations
of Gates it appears to be of the order of 1 per cent. This
would give about two 8-chromosome pollen grains in 400 or
0·5 per cent. If the frequency of this irregularity in the
megaspore mother-cells is the same, about 1 per cent. of lata
mutations should be anticipated (c f. p. 527). The relative
numbers of the two types of megaspores produced by lata
may be estimated from the number of lata plants occurring

in lata × Lamarckiana.   The number of latas in this
cross, as shown by the cultures of de Vries (1913, p. 245),
fluctuates widely, from 4 per cent. to 45 per cent., though
most commonly falling near 20 per cent.   No doubt the
environmental conditions at the time when the meiotic
divisions in the megaspores are taking place, or the physio-
logical condition of the mother plant at this time, determine
whether the percentage of latas in the cross will be large or
small.   It is probable that in a similar way environmental
circumstances control in a measure the wide variations in the
percentage of the types, such as occur in many Œnothera
hybrids.

If functioning megaspores with 7 and 8 chromosomes
respectively, were formed with equal frequency and had equal
capacities for development, the cross lata × Lamarckiana
should yield 50 per cent. of each type.   But evidently the
8-chromosome megaspores have less prospect of functioning,
since the percentage of latas falls usually to 20 per cent.
and sometimes to 4 per cent.   In certain cases, however, the
number of latas from a cross rises even above 50 per cent.
Thus lata × biennis yielded 53 per cent. in 258 plants, and
lata × biennis cruciata gave 60 per cent. lata (de Vries,
1913, p. 251).   Again, de Vries found that lata × gigas
gave an offspring about half of which were intermediate
between lata and gigas, and half intermediate between
Lamarckiana and gigas.   It seems probable that the
former has 22 and the latter 21 chromosomes.

The other hereditary peculiarities of lata may be similarly
explained by the presence of the extra chromosome.   Thus,
Lamarckiana × Hookeri or its reciprocal gives the twin
hybrids laeta and velutina in F_1 (de Vries, 1913, p. 131),
while lata × Hookeri (l. c., 252), produces, in addition to
these two types, laeta-lata and velutina-lata.   There
can be no doubt that the last two types, which occur in 8–33
per cent. of the offspring, owe their appearance to the presence
of the extra chromsome.

Certain other mutants indicate by their hereditary behaviour

that they may also have aberrant chromosome numbers, but this has not yet been proved, except in gigas. It is also to be expected that some of the offspring of semilata or of fertile lata plants contain 16 chromosomes, but such plants have not yet been observed.

As already pointed out, lata and semilata form an almost continuous series, all, however, having certain features in common, and all having 15 chromosomes. They may also, of course, possess various minor characters in addition. The presence of red or white midribs on the leaves, e. g., appears to be a simple Mendelian character-difference. But the relation between lata and semilata is not at present clear. The variability of the lata-semilata series may depend upon the fact that the extra chromosome belongs to a different pair in different cases. Since there are seven pairs of chromosomes, we should then expect seven more or less distinct lata-like types. But there is at present no evidence that the plants having 15 chromosomes can be divided in this way.

An equally plausible hypothesis would be that any chromosome can constitute the extra one in any member of this series of plants, and that the external variability exhibited by them depends upon certain chromosomes not being distributed whole during meiosis. Though such processes have already been described, there are difficulties also with this view, for it involves the rather unlikely assumption that the chromosomes of Œnothera are undifferentiated in their potentialities. The cause of this external variability, and particularly of the difference in fertility, leaf shape and habit between lata and semilata therefore remains for the present obscure, though semilata stands midway between lata and Lamarckiana.

What has been made clear, however, is a constant association between the extra chromosome and the lata-like constellation of external characters, and we believe this to be the first case in plants in which such a relationship has been shown to exist. A comparison with the sex chromosomes in

animals, and particularly with the cases where the females
have one more chromosome than the males, is at once sug-
gested. In such cases as Anasa tristis among insects, the
males have an odd, unpaired or heterotropic chromosome,
while in the females this chromosome is present in duplicate,
and therefore has a mate. In spermatogenesis, half the sperms
receive the odd chromosome and half do not, while all the
eggs contain this chromosome. Hence eggs fertilised by a
sperm containing the odd chromosome produce females in
which this chromosome is present in duplicate, and eggs
fertilised by the other class of sperm produce males with an
unpaired chromosome.

The case of Œ. lata differs from this in several respects.
In the first place, the extra or fifteenth chromosome is not a
duplicate, but a triplicate of a pair already present. And in
the second place this difference in chromosome content is
associated, not with sex (unless the male-sterility of lata be
significant in this connection), but with a difference in the
foliage and various other features of the plant. It is possible
to say, however, that in these plants the extra chromosome,
when present as the triplicate of a pair, is constantly associated
with the development of certain foliage characters, in the
same way that the accessory chromosome when present in
duplicate in certain insects is constantly associated with the
development of the female sex characters.

In certain respects the extra chromosome resembles more
closely the supernumerary chromosomes described by Wilson
(1909, 1910) in Metapodius. Without detailing the rather
complicated facts in this genus of Hemiptera, it may be said
that the typical or fundamental number of chromosomes
is 22 in both males and females, including 18 auto-
somes, two m-chromosomes, and two idiochromosomes or
sex chromosomes. Of the latter, the females possess two large
ones and the males a large and a small. Variations from
this condition occur, and though the chromosome-number for
each individual is practically constant, yet in different
individuals of, e. g., M. granulosus, the number ranges

from 22 to 27. The increase in number is brought about in nearly all cases by duplication of the small idiochromosomes. This is shown not only by the sizes, but particularly by the behaviour of the supernumeraries during the reduction divisions. That is, they retain their condensed form during the growth stages of spermatogenesis, forming a compound body with the idiochromosomes. Like the latter they divide as separate univalent chromosomes on the heterotypic spindle, on which they usually occupy a characteristic position, while the other chromosomes undergo a reduction division. In the homotypic division they are somewhat erratically distributed, and this results in variations in the numbers of supernumeraries in different individuals of the next generation. By repetition of this process the number of supernumeraries may sometimes become as great as six.

As regards the origin of the supernumeraries, Wilson has observed cases in the spermatogenesis of 22-chromosome individuals in which both idiochromosomes passed into the same germ-cell in the second, which is for them the reduction, division. Such a sperm, if functional, would produce an individual having 23 chromosomes, including one supernumerary; the later duplications to give as many as five or six supernumeraries, apparently all arise through similar irregular distributions of these particular chromosomes in later generations. These facts constituted a remarkable confirmation of the theory of genetic continuity of the chromosomes.

Thus the supernumeraries in Metapodius owe their origin to the same cause as does the extra chromosome in Œ. lata, namely, to an irregular meiotic distribution of chromosomes. But while plants having the extra chromosome are easily recognisable by their external characters, it appears that insects having even five or six supernumerary chromosomes are indistinguishable from the others even in size. This indicates that the supernumeraries are inactive, which is in harmony with the general view of Wilson that the small idiochromosome, or Y-element, of which they are duplicates, takes no active part in the determination of sex.

The numerous types of meiotic irregularity described in this paper call for no remarks except to point out that not only may they lead, in the comparatively rare instances when such cells survive, to new numbers of chromosomes, but they might result in the perpetuation of half-chromosomes or fragments of chromatin in later cell generations. This, we believe, would be a source of external variability in the plants, and all such alterations in nuclear content should be looked upon as germinal changes.

The Chromosomes in Ontogeny.—The statement is sometimes made that variations in chromosome number are of no greater significance than any other variations, and that in a lily, for example, deviation from 24 as the chromosome number is no more significant than fluctuation of the stamens around 6, the usual number. It is obvious, however, that a fundamental difference exists between the nuclear constitution of any organism, which is determined at the time the pronuclei unite in fertilisation, and the external characters of the organism, which appear later. In other words, the chromosome content is practically the only visible structure which is of primary significance, because it is transmitted as such from the previous generation, while all external features arise secondarily through the interaction of nucleus and cytoplasm as the organism develops.

Finally, the lata and semilata forms furnish in their origin a case of mutation par excellence, the essential germinal change occurring when a chromosome is distributed to the wrong germ nucleus. The germinal change constituted by this irregular or exceptional meiotic division leads to the development of an individual whose cells have a 15-chromosome instead of a 14-chromosome content. The organism, therefore, develops along a new line of stability, though that stability cannot be fully maintained in the offspring, because in reduction there is an odd or unpaired chromosome. In the lata rubricalyx individual above described, the contrast between the usual processes of hybrid inheritance and the exceptional changes constituting a mutation is

particularly clearly drawn. And in the light of these facts we are forced to contrast sharply the ordinary hybrid behaviour, in which the characters contained in the parents reappear in the offspring in various re-combinations and blends, with mutations, in which a germinal change occurs, leading, as with Galton's polyhedron, to a new condition of stability in the organism. Tetraploidy and the presence of the extra chromosome are both cases in which the occurrence of such germinal changes is proven beyond doubt.

Obviously, then, tetraploidy and the occurrence of mutants with an extra chromosome are both processes which are not Mendelian in character, and they are of much importance at the present time as proving cytologically the essential correctness of the conception of germinal changes or mutations. When only the external characters are considered, it may be difficult or impossible to prove with any finality that the new characters are really progressive or that they do not represent merely recombinations of previous characters. But the nuclear structure furnishes incontrovertible evidence.

In conclusion, we are indebted to Prof. J. B. Farmer, F.R.S., for the examination of a number of our critical preparations.

Summary.

This paper contains a study of the lata and semilata series of mutations in Œnothera. Twenty-one such plants in cultures from various sources were examined and 15 chromosomes have been found in every case, though the mutants were derived from 14-chromosome races. The races in question were from such diverse sources as Sweden, Hungary, Madrid, Birkenhead, the cultures of de Vries, and the offspring of certain hybrids.

Especial interest attaches to a 15-chromosome mutant called Œ. lata rubricalyx, which appeared in the F_2 of Œ. mut. rubricalyx × Œ. grandiflora, and which combined the foliage and habit of lata with red pigmentation inherited from rubricalyx. Similarly the race of Œ. biennis L. from

Madrid contained a lata mutation, having lata-like foliage
and the small flowers of biennis.

These cases, together with the previous work, prove that
the peculiar characters of lata and semilata are constantly
associated with the presence of 15 chromosomes, even when
combined with other characters derived by inheritance from
14-chromosome individuals.

These mutants with 15 chromosomes have acquired the
extra one by the occasional distribution of two chromosomes
of a pair to the same daughter-nucleus in the reduction divi-
sion, such occurrences having been discovered by one of us in
1908.   The inconstancy of lata and semilata is explained
by the behaviour of the extra chromosome, as is also the fact
that lata × Lamarckiana gives both parent types in the F_1,
since lata produces some germ-cells having 7 and some
having 8 chromosomes.   The proportion of latas in the
cross varies widely, from 4 per cent. to 45 per cent., the
percentage being determined by the number of 8-chromosome
germ-cells which mature.   The fluctuation in this ratio is
probably caused (1) by the environmental conditions at the
time the germ-cells are being developed, and (2) by the
physiological condition of the mother plant at this time.   The
various other hereditary peculiarities of lata and semilata
are also explained by the presence of the extra chromosome.

The cause of the variability in the lata-semilata series
of forms is at present obscure, but it may depend on the
irregular distribution of portions of chromosomes during
meiosis.

The extra chromosome is associated with the foliage and
habit of lata or semilata in the same way that one of the
sex chromosomes is associated with sex in such insects as
Anasa tristis, with this difference, that in Œ. mut. lata
or semilata the extra chromosome is a triplicate of a pair
already present, while in these Hemiptera the presence of
the accessory chromosome in duplicate is constantly associated
with the female sex.

The extra chromosome in Œnothera resembles more closely

in some respects the supernumerary chromosomes described by Wilson in Metapodius. The latter arise in a similar way, by the passing of both idiochromosomes into the same nucleus in meiosis, but they are duplicates of the Y-element in sex determination, and as such apparently have no effect on the external characters of the organism.

The view is expressed that the initial nuclear difference, in having 15 instead of 14 chromosomes, determines the change in the external characters in the Œ. lata-semilata series of mutants. And the fact is emphasised that the chromosome number is a primary character of any organism, originating in the fertilised egg, while the external features are all secondary in development.

A number of different types of meiotic irregularity are described in the lata and semilata mutants. These are to be looked upon as germinal changes, though for the most part their products degenerate. The irregularities studied include the division of a chromosome (probably the extra one) on the heterotypic spindle, and various other types of fragmentation and degeneration of chromosomes, enumerated on p. 549.

The fact that lata-like mutations appear sporadically in various races, species and interspecific hybrids of Œnothera, combining in some cases the hybrid characters of their ancestors with those of lata, shows, as does the presence of the extra chromosome, that mutations and Mendelian hybrids should be contrasted; for they owe their origin to distinct causes, the former to a germinal change, the latter to a redistribution of the parental characters.

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Note.—At the request of Sir Ray Lankester I have added the following note regarding the definition of mutation and the classification of various other processes which may be grouped under the general term "variation." Of course, it will be understood that there are not hard and fast lines of limitation between these processes. They probably shade into each other just as do many species. Nevertheless, definitions

are not only of great value for clarifying our conceptions, but they are necessary for accuracy in discussion and for obtaining a picture of the relationships between the various types of change. It is with these ideas in mind that the present classification of such processes is offered, since these terms have been and are being used in many senses by various authors.

It has been urged that the term "mutation" should be used only in the palæontographical sense employed by Waagen, but such limitation of the term seems undesirable for a number of reasons. In the first place Waagen was not the first to make use of the term, but it was employed by botanists in a sense more nearly approaching its present usage long before Waagen's time. Further, no law of priority with regard to such terms exists, and it is undesirable that any such law should be established. Moreover, the application of such a law would be impossible, for the significance of terms of this kind is of necessity constantly undergoing modification with the increase in our knowledge of the phenomena of heredity and variation. In the few cases where there is a possibility of confusion arising, any difficulty may be overcome by defining one's terms or by employing a more specific phraseology. Thus it would be easy to speak of the "mutations of Waagen" in contrast to the "mutations of de Vries."

In the present state of our knowledge it seems desirable to define a mutation as a discontinuous germinal change arising from a physical or chemical alteration in the structure of the organism (in micro-organisms) or of one or both of the germ-cells (in higher organisms) which produce a new individual, or from such a change arising in certain cells elsewhere in the life-cycle of the organism, this change being capable of complete inheritance, at least in some of the offspring, though reversion may occur in the others.

In short, a mutation is a germinal change which is completely inherited in a portion at least of the offspring, though the others may show reversion.

Fluctuations we may define by contrast as continuous changes arising from the effects of environment or nutrition which are only partly inherited and hence which show Galtonian regression, the whole population forming a continuous series in regard to a fluctuating character.

Mutations and fluctuations are thus contrasted in several particulars. As regards inheritance, they are both inherited, but in different degrees and in different ways. In this view I am in agreement with Professor E. B. Poulton (see "The Term 'Mutation,'" 'Proc. Section D, British Association,' 1913), and regard it as a mistaken conception to hold, as has frequently been done, particularly by the Mendelian school, that mutations are inherited while fluctuations are not. In the discussion following Professor Poulton's paper, I suggested that the terms partial and complete inheritance should be used with reference to fluctuation and mutation respectively. That is, mutations are completely inherited either in all the offspring, or at least in a portion of them with reversion in the others; while fluctuations are partially inherited in varying degrees in the offspring, and thus form a continuous graded series ranged on one or both sides of the parental condition according to the place occupied by the parent organism in the original series.

It is probable that in addition to these partially inherited fluctuations there are also non-inherited or somatic fluctuations.

Mutations are also contrasted with Mendelian metathesis or recombinations. The former deal with the origin of new and discontinuous characters, while the latter are concerned merely with the combinations and permutations of such characters as have already appeared. Obviously, then, Mendelism sensu stricto is not concerned with the origin of new unit-characters. The sudden origin of a character constitutes a mutation; its inheritance may be Mendelian or otherwise, but is in any case a process distinct from the change which brought about its origin. In this aspect mutation deals with discontinuous origins and Mendelism with discontinuous inheritance.

There are two methods of studying the nature of any particular mutational change. One is the comparison, cytologically as well as morphologically and chemically, of the mutant with its parent; the other is by studying the inheritance of the mutation in its offspring and in crosses with its parent race. When both these methods are used together they supplement each other, and the cytological investigation of mutants in particular has served as a useful check on Mendelian speculation which would explain all mutants in terms of a single idea.

In the following table I have attempted to classify the various types of mutational change. Though the classification is necessarily incomplete, it serves to indicate the variety of types of mutation which are now known. Examples of each type are given from the many instances available in the recent literature. The cases have been selected both from plants and animals, and the name of the organism with the name of the discoverer or investigator of the mutation is given in each case.

Near the borderland between mutations and fluctuations, and partaking somewhat of the nature of both, are such variations as variegation of foliage (e. g. Acer striatum variegatum Godron), and striped flowers which, according to Vilmorin, originate through partial reversion from white varieties. The "ever-sporting varieties" described by de Vries are also perhaps to be placed here.

Dobell (1913), in his recent useful review of the work on mutation in Trypanosomes and Bacteria, uses the term mutation "to denote those heritable modifications which have been induced in various ways in various micro-organisms." This appears to be essentially the same as my conception of a "germinal change." Again, by mutation in Bacteria he understands that "in a given race individuals may occur which differ from their fellows in their genetic constitution." In certain cases these differences seem to be acquired not suddenly, but by several small successive steps.

It will thus be seen that a host of phenomena of widely

MUTATIONS.

Experimentally induced {
Leptinotarsa, Tower.
Phascum cuspidatum, É. and É. Marchal.
Aspergillus niger. Schiemann.
Trypanosoma brucei, Werbitzki.
Bacillus prodigiosus, Wolf.
}

Mutations.→Produced by wounding—Zea mays pennsylvanica var. præcox, Blaringhem.
var. pseudo-androgyna, Blaringhem.

"Spontaneous."—Producing a series of new forms {
Œnothera, de Vries.
Drosophila, Morgan.
}

In asexual micro-organisms:
Bacillus coli, Barber.
Bacterium coli mutabile, Massini.

Originating through a physical or morphological change:
Œ. gigas, de Vries.
Œ. semigigas, Stomps.
Œ. lata, de Vries.
Œ. semilata, de Vries.
Trypanosoma evansi, etc., Laveran and Roudsky.

In sexual higher organisms: Œnothera.

Completely inherited:
Œ. gigas.
Œ. rubrinervis.

Originating through chemical or physiological change:
Œ. rubricalyx, Gates.
Bacillus coli - typhosus, Twort.
Trypanosoma lewisi? Gonder.

Originating as a heterozygous dominant character:
Œ. rubricalyx.

Originating through loss or latency of a character:
Melasoma scripta, McCracken.
"Cretin" sweet pea, Bateson and Punnett.
Peromyscus leucopus noveboracensis mut. albidus, Castle.

Completely inherited recessive character:
Œ. nanella.
Œ. brevistylis.

Originating through modification of a character:
Capsella bursa-pastoris mut. Heegeri, Solms-Laubach.

Inherited usually in a small percentage:
Œ. lata.
Œ. semilata.

Originating through reversion: Zea mays tunicata, East.

Originating.

In the wild.
Peromyscus leucopus noveboracensis mut. albidus, Castle.
Potentilla verna mut. monophylla, Domin.
Helianthus lenticularis mut. coronatus, Cockerell.

In cultivation.
Primula officinalis mut. horticola, Domin.
Melandrium album with small leaves, Baur.

Originating

In fertilisation or subsequently:

As vegetative mutation.

Through segregation in somatic cells of heterozygous races:
Solanum tuberosum. East.
Veronica longifolia × V. l. alba, deVries.

Œ. gigas?
Periclinal and sectorial chimeras?

In pure races:
Phaseolus vulgaris. Johannsen.

Through change in somatic cells from homozygons to heterozygous condition.
Mirabilis Jalapa variegata. Correns.

In meiosis:
Œ. lata.
Œ. rubricalyx.
Œ. biennis semigigas.
H. lenticularis mut. coronatus, Cockerell.

Originating

In crosses:
Many probable cases.
Bombyx mori, Toyama.
Antirrhinum majus, Baur.

In pure races:
Hordeum distichum, Kiessling,

New dominant characters:
Œnothera rubricalyx.
Zea mays, albinistic ear, Collins.
Helianthus lenticularis coronatus, Cockerell.
Primula sinensis, giant, Keeble.

New recessive characters:
Many dwarf varieties,
Many white varieties of flowers, and albino animals.

differing types are to be classed under the general term " mutation," i.e. a germinal change originating through a relatively sudden and marked alteration in the character of a particular somatic or germ cell. R. R. GATES.

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EXPLANATION OF PLATES 35, 36 AND 37,

Illustrating Dr. R. Ruggles Gates and Miss Nesta Thomas's
    paper on " A Cytological Study of Œnothera mut. lata
    and Œ. mut. semilata in Relation to Mutation."

[All the figures were drawn with the camera lucida.   Figs. 1–31, 35–
54, under a 2 mm. apochr. Hom. imm. Zeiss, N.A. 1·40, with comp. oc.
18 (× 2900) ; figs. 32–34 under a 3 mm. apochr. Hom. imm. Zeiss, N.A.
1·40 with comp. oc. 4 (× 500).]

Somatic Mitosis.

x indicates the odd or unpaired chromosome.

PLATE 35.

Œ. semilata.

Fig. 1.—Prophase showing 15 chromosomes and nucleolus.

Fig. 2.—Prophase showing 15 chromosomes and two nucleoli.

Fig. 3.—Metaphase. Polar view showing 15 chromosomes.

Fig. 4.—The same, one chromosome having a curious prolongation.

Fig. 5.—The same, one chromosome much smaller than the other 14.

Fig. 6.—Metaphase. Polar view, showing the paired arrangement of the chromosomes.

Fig. 7.—Metaphase. Similar group to that shown in fig. 6. One of the chromosomes is dumbbell-shaped.

Œ. biennis lata.

Fig. 8.—Metaphase showing group of 15 chromosomes, one of which is very much shorter and one rather longer than the average chromosome.

Fig. 9.—A similar group of 15 chromosomes, one of which has a small piece constricted from it.

Œ. lata.

Fig. 10.—Polar view of metaphase showing 15 chromosomes, one of which appears very much shorter and thicker than the remaining 14.

Fig. 11.—Polar view of metaphase showing exceptionally strong pairing in a group of 15 chromosomes.

Œ. lata.

Fig. 12.—An exceptional group of 15 chromosomes, in which 9 show a distinct light area, usually near their middle points.

Fig. 13.—A similar group of 15 chromosomes, all of which show this light area.

Œ. semilata.

Fig 14.—Metaphase in polar view. Seventeen or 18 chromosomes, some of which are splitting at one end only. See text.

Œ. lata.

Fig. 15.—Metaphase in polar view, showing 15 chromosomes. A strongly paired group, two chromosomes of one pair with a precocious split.

Meiotic Divisions.

Œ. rubricalyx.

Fig. 16.—Typical heterotypic spindle in profile, showing 14 chromosomes somewhat irregularly scattered on the equatorial plate.

Fig. 17.—Polar view of heterotypic spindle, showing 14 chromosomes lying in several planes.

Œ. lata rubricalyx.

Fig. 18.—Diakinesis, showing 15 chromosomes and two small nucleoli.

Fig. 19.—Heterotypic spindle in profile, with the 15 chromosomes irregularly arranged. A great variation in the size of the chromosomes may be observed.

Fig. 20.—Polar view of heterotypic nucleus at the metaphase stage, showing 15 small chromosomes lying in vacuolate protoplasm.

Fig. 21.—Metaphase group of 15 chromosomes. The cells show signs of degeneration.

Fig. 22.—Typical anaphase of heterotypic division. The spindle was cut and only 11 chromosomes are seen in the section, but these all show the longitudinal fission.

Fig. 23.—Interkinesis. Seven split chromosomes and a nucleolus are contained in the nucleus. An eighth chromosome may be seen just outside the nuclear membrane.

Fig. 24.—Polar views of the two groups of chromosomes in the homotypic metaphase. Each group contains 7 whole chromosomes and a $\frac{1}{2}$ chromosome. Several of the whole chromosomes show the longitudinal split, and in the right-hand group the two halves of one chromosome are rather widely separated from each other. The distribution here represented has resulted from the longitudinal division of a chromosome, presumably the extra one, on the heterotypic spindle. S indicates the two halves of this chromosome.

Fig. 25.—Homotypic metaphase in polar view. Cell and chromosomes exceptionally small. The left-hand group has clearly 7 whole and one $\frac{1}{2}$ chromosomes, while the right-hand group consists of 6 whole chromosomes, a $\frac{1}{2}$ chromosome and a small fragment. Not including the fragment there are 14 chromosomes present in the two nuclei. The fifteenth may be seen in the protoplasm between the two nuclei, having failed to enter the right-hand nucleus. S indicates the halves of the chromosome which split in the first division.

Fig. 26.—Profile view of typical homotypic spindle.

Fig. 27 a and b.—Two homotypic anaphase spindles in profile, from a

single mother-cell.   *a.* There are 6 daughter-chromosomes moving towards either pole, and at the equator two fragments which together are equivalent to a single daughter-chromosome.   *b.* Eight daughter-chromosomes are moving towards either pole of the spindle, and in the centre are two small fragments.   Apparently these four fragments are formed by the two halves of the extra chromosome which split at the heterotypic metaphase.

Fig. 28.—Anaphase of homotypic division, showing 8 daughter-chromosomes going to either pole of one spindle.

PLATE 36.

Fig. 29.—Late anaphase of the homotypic division, showing the 4 chromosome groups of the tetrad.   The upper pair contains 7 chromosomes and the lower pair 8 chromosomes, in each group.

Fig 30 *a* and *b.*—Homotypic mitosis in two sections.   In *b* 6 chromosomes are seen at one pole of the spindle, the remaining 2 being in section *a* ; 8 are approaching the other pole of this spindle. In the second spindle there are 6 daughter-chromosomes at one pole and 4 at the other.   The 4 remaining daughter-chromosomes on this spindle are degenerating in the equatorial region.

Fig. 31 *a* and *b.*—Late homotypic anaphase.   *a.* On the lower spindle each anaphase group contains 8 chromosomes.   On the other spindle each group has 7 chromosomes, section *b* showing 5 chromosomes close together and two displaced, one of the latter (*c*) having also been cut by the knife so that the greater part of (*c*) appears in section *a.*

Fig. 32.—Degenerating anther.   Mother-cells connected by broad bands of cytoplasm.   Cytoplasm scanty.   No tapetal cells differentiated.

Fig. 33.—Mother-cells degenerating.   Nuclei as dark chromatic masses passing through the walls.   Tapetum not differentiated.

Fig. 34.—Another type of degeneration, tapetum not differentiated. The mother-cells are in contact, the chromosomes short and scattered, and passing through the walls.

Fig 35.—A mother-cell in another condition of degeneration.   The cytoplasm a coarse, dark, highly vacuolate reticulum.   The nucleus has disappeared or is represented by the dark mass at the centre of the cell.

Œ. biennis lata.

Fig. 36.—Normal polar view of heterotypic metaphase, showing 15 chromosomes.

Fig. 37 *a* and *b.*—Profile view of heterotypic spindle in two sections

showing 15 chromosomes. Some of the chromosomes are losing some of their chromatin, a trail of which is left on the spindle as the chromosomes pass to the poles.

Fig. 38.—Normal metaphase of homotypic division, one spindle in polar view and one in profile, showing the 8–7 distribution of chromosomes.

Fig. 39.—Normal homotypic metaphase, oblique view showing 7–8 distribution of chromosomes, in several of which the split may be seen.

Fig. 40.—Polar view of the two homotypic metaphase groups in a mother-cell. The lower group contains 8 whole and one $\frac{1}{2}$ chromosomes, though the former vary in size. The upper group contains 6 whole and one $\frac{1}{2}$ chromosomes. S indicates the halves of the chromosome which split at the heterotypic metaphase.

Fig. 41 a and b.—Irregular distribution of chromosomes. Polar view of one group of chromosomes at the homotypic metaphase, showing 9 chromosomes. The second spindle, having only 6 chromosomes, is seen in profile in two sections.

PLATE 37.

Œ. near Lamarckiana.

Fig. 42.—Heterotypic spindle in profile, showing 14 chromosomes. Two of the chromosomes are leaving a trail of chromatic substance as they move towards the pole.

Fig. 43.—Normal homotypic metaphase with two groups of 7 chromosomes.

Fig. 44.—Irregular homotypic metaphase. In the left-hand group there are probably 7 whole and one $\frac{1}{2}$ chromosomes, while the right-hand group contains apparently 6 whole and one $\frac{1}{2}$ chromosomes. This indicates that one of the 14 chromosomes has split on the heterotypic spindle, though there is great variation in the size of the chromosomes.

Fig. 45.—Late anaphase of second division, showing the four anaphase groups, each with 7 chromosomes.

Œ. mutant resembling lata.

Fig. 46.—Normal heterotypic spindle in profile, showing 15 chromosomes scattered on the spindle.

Fig. 47.—Irregular heterotypic anaphase with 9 chromosomes moving to one pole of the spindle and 5 to the other. The fifteenth chromosome has been pulled into two parts, leaving a chain of chromatin between them.

Fig. 48. — Similar to fig. 47, but here 6 whole chromosomes are moving to one pole and probably 8 to the other. In the former group is a chromosome which has left behind a long trail of chromatin, while the latter group contains a small fragment of chromatin. At the periphery of the spindle is a nucleolus.

Fig. 49.—A similar case of degeneration and loss of chromatic substance ; two chromosomes are behaving as in figs. 47, 48, and are losing their chromatic substance. The spindle is cut, and the full number of chromosomes is not present. The chromosomes are also much smaller than normal.

Fig. 50 *a* and *b*.—Late homotypic prophase of the two nuclei in a pollen mother-cell. In fig. 50 *a* there are 7 chromosomes just going on the spindle, while the sister nucleus in fig. 50 *b* shows 8 chromosomes. Nearly all these chromosomes show the longitudinal split.

Fig. 51.—One nucleus of a pair at the homotypic metaphase, showing 8 chromosomes in a group, and a ninth which is outside the nucleus and will probably degenerate.

Œ. lata.

Fig. 52.—Normal heterotypic spindle in profile, showing 15 chromosomes On the periphery of the nucleus are two nucleoli which have persisted up to this late stage.

Œ. lata to semilata.

Fig. 53.—Metaphase of one of the nuclei of the homotypic division, showing 7 chromosomes, the two halves of one of which are widely separated.

Fig. 54.—Homotypic metaphase, showing both nuclei in polar view. In the left-hand group are $7\frac{1}{2}$ chromosomes, and in the right-hand group $6\frac{1}{2}$ chromosomes. Between the groups are fragments which together constitute the fifteenth chromosome, which was probably left behind on the heterotypic spindle.

The half-chromosomes are marked *S*

On the Development and Morphology of the Mandibular and Hyoid Muscles of Mammals.

By

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With Plates 38–45 and 9 Text-figures.

In the course of a paper on the morphology of the muscles of the head in Vertebrates published in this Journal two years ago, I gave a short description of those of the mandibular and hyoid segments in the rabbit. There is great difficulty in distinguishing muscle-Anlagen from surrounding mesoblast in the early stages of developing Mammals, and, not quite satisfied with some of the statements made, I have re-investigated the phenomena. The inquiry has been much facilitated by Prof. J. P. Hill, who very kindly lent me sections of Dasyurus viverrinus—an animal which is born with two masticatory muscles only and an incudo-meckelian joint, and in which the development of typical mammalian muscles and of a squamoso-mandibular joint takes place after birth. These changes can be easily followed, and enable one to interpret the more obscure phenomena occurring in Mammals with a longer intra-uterine development. Other Mammals have also been investigated, viz. pig, rabbit, Phoca vitulina, Halichœrus grypus, Bradypus marmoratus, Dasypus novemcinctus, Manis pentadactyla, Didelphys aurita, Echidna aculeata, Ornithorhynchus.

The first investigator of the development of the masticatory muscles was Reuter, who stated that in the pig their Anlage is first visible in embryos of 16 mm. Nacken-Steisslänge in

the form of an inverted **Y**, the two limbs of which lie on either side of the lower jaw. The temporal muscle develops from the upper limb, the masseter from the lower external limb, and the two pterygoids from the lower internal limb. He did not mention the tensor tympani or the tensor veli palatini. Eschweiler described the development of the tensor tympani and tensor veli palatini in the pig. He found the first indication of the muscles in embryos of 15·75 mm. Scheitel-Steisslänge, when the masticatory musculature appears in the "Blastemsäule" which contains Meckel's cartilage as its nucleus. The Anlagen of the tensor tympani and the malleus portion of Meckel's cartilage form at first an indivisible mass. The differentiation of the muscle-Anlage to muscle occurs in embryos of 20·5 mm. It forms the aboral end of the "Blastemsäule," which, orally, forms the masticatory muscles. On the medial side of the Anlage of the masticatory muscles develops the Anlage of the tensor veli palatini; its aboral end is gradually lost in connective tissue, which is continuous, aborally, with the Anlage of the tensor tympani (i. e. the two muscles do not overlap). The Anlage of the tensor tympani shifts aborally, whilst its development into muscle takes place in the reverse direction. In later development the oral end of the tensor veli palatini spreads into the velum, and the tensor tympani gains an attachment to the labyrinth capsule.

Lewis (1910) gave an account of the development of the masticatory muscles in man, which is very similar to that given by Reuter in the pig. The chief point in which he differed is that "in a 14 mm. embryo the Mm. tensor tympani and tensor veli palatini are to be recognised and are connected with the ptyerygoid mass from which they probably arise.'"

In the following account of the development of the mandibular and hyoid muscles, those of the mandibular segment are first described and subsequently those of the hyoid segment. On account of the relationship of the anterior digastric to the posterior digastric, it is convenient to consider the depressor mandibulæ anterior and anterior digastric

(which are proliferated from the intermandibularis) with the hyoid muscles.

In stage A (just born) of Dasyurus (figs. 1 and 2), there is a cartilaginous ala orbitalis, orbito-parietal commissure, and parietal-platte, continuous with one another. The ala temporalis forms a ventro-lateral process of the presphenoid region of the chondrocranium; its free extremity turns forward. There is no line of demarcation between the processus alaris and ala temporalis. The incus is a precartilaginous mass. Meckel's cartilage is a continuous cartilaginous structure, and has not divided into malleus and portion in front. The Anlage of the mandible is a group of cells dorsal to and extending down a little distance on the outside of Meckel's cartilage. In this Anlage, dorso-lateral to Meckel's cartilage, ossification is just visible in the region of attachment of the lateral muscle; anteriorly it is more marked and extends forward almost to the front end of the cartilage. Two masticatory muscles are present, medial and lateral. The medial muscle arises from the ala temporalis; its most anterior fibres pass downwards and outwards to the inner surface of Meckel's cartilage, whilst the succeeding ones pass more and more obliquely backwards to the bar; the hindmost fibres are attached as far backwards as the malleus portion; the fibres form one continuous sheet. The lateral muscle—the front end of which is anterior to that of the medial muscle—arises from the orbito-parietal commissure, passes downwards, and is attached to the upper border of the Anlage of the mandible. Both muscles consist of cross-striated muscle-fibres. The third division of the fifth cranial nerve passes downwards between the two muscles, giving off the ramus medialis to the medial muscle and the ramus lateralis to the lateral muscle. The intermandibularis forms a ventrally curved transverse sheet between Meckel's cartilages; it has a median raphé, and is attached laterally to the inner surface of the cartilage. The mylohyoid nerve passes down on its outer surface.

In stage C (figs. 6 to 11) the medial muscle has separated into anterior and posterior portions. The former is the

internal pterygoid, the latter the common Anlage of the
tensor veli palatini and tensor tympani. The internal ptery-
goid retains its origin from the ala temporalis, whilst the upper
end of the Anlage of the tensor veli palatini and tensor
tympani has grown inwards beneath the ala temporalis and
is attached to the (mammalian) pterygoid bone, which has
now developed behind and in continuity with the palatine
bone. The coronoid process of the mandible is beginning to
form, and the insertion of the lateral muscle has spread down
a little on its outer side. Cells proliferated downwards and
backwards from the anterior of these muscle-fibres on the
outside of the mandible form the beginning of the masseter
muscle (fig. 6).

In stage D (figs. 14 to 18) a cartilaginous bar—the
processus ascendens alæ temporalis—has appeared, extending
from the upper lateral edge of the ala temporalis to the orbito-
parietal commissure; it is a cartilaginous thickening of the
anterior edge of the membrana obturatoria covering in the
spheno-parietal foramen. The Anlage of the tympanic bone
has appeared as a straight rod external to the hinder part of
Meckel's cartilage; its anterior end is overlapped by the
posterior end of the mandible. The origin of the lateral
muscle—hitherto confined to the orbito-parietal commissure—
has now additionally spread downwards, so that the muscle
arises from orbito-parietal commissure, the membrana obtura-
toria and the processus ascendens. The coronoid process of
the mandible has extended further upwards and the inner
fibres of the lateral muscle are attached to its medial side.
The Anlagen of the squamous and malar bones are formed,
and the masseter in part arises from them. The anterior
digastric is being proliferated from the hinder part of the
intermandibularis.

In stage E (figs. 20 and 21) a condylar process has formed
as a slight elevation of the hinder end of the upper edge of
the mandible; and the external pterygoid muscle, of which
there is no trace in stage D, is differentiated from the lower
posterior edge of the lateral muscle. It consists of muscle-

cells which are much smaller in size than those of the rest of the lateral muscle, and is probably proliferated from it, and not formed by change of direction of already existing muscle-fibres. The external pterygoid muscle takes origin from the lower end of the ascending process of the ala temporalis and passes outwards to the condylar process of the mandible. The lower anterior fibres of the lateral muscle, which now forms the temporal, arise from the ascending process in front of the origin of the external pterygoid.

In stage F (figs. 22 and 23) there is a downward and backward growth from about the middle of the tympanic bone. The goniale bone[1] is also formed; it lies dorsal-median to the tympanic bone, between it and Meckel's cartilage; its anterior end extends slightly further forwards than does the tympanic, and its posterior end further back, underlapping the malleus portion. The origin of the temporal muscle has extended backwards so that it additionally arises from the parietal-platte. The common Anlage of the tensor veli palatini and tensor tympani is beginning to separate into those muscles, and the insertion of the latter has shifted down the side of the malleus portion of Meckel's cartilage to its manubrial process. The tensor veli palatini is inserted on the inner side of Meckel's cartilage and does not reach the tympanic bone lying on the other side of Meckel's cartilage. Fig. 39, taken from a 10 mm. specimen of Didelphys aurita, shows a little more advanced stage of the same condition.

In stage H (figs. 24–27) the parietal bone is formed outside the orbito-parietal commissure and parietal-platte, and the temporal arises from it.

The alisphenoid bone is formed on the outer and upper sides of the ala temporalis, and upwards round the processus ascendens, which is also involved in the ossification. The bone extends in front of and behind the process. The lower anterior fibres of the temporal, the external pterygoid, and the upper head of the internal pterygoid correspondingly

[1] I use Gaupp's nomenclature. Palmer has recently homologised it with the supra-angulare, though without any discussion of Gaupp's views.

arise from this bone, and the upper fibres of the temporal also from the parietal bone. The external pterygoid has two heads, separated from one another by the buccal nerve. The lower portion of the temporal is beginning to be separated off as the zygomatico-mandibularis. The internal pterygoid has become partially separated into two portions, one head—the original one—arising from the alisphenoid bone, the other from the palate bone; the latter head, in stage J, additionally arises from the pterygoid bone. The proximal portion of the tensor tympani, i. e. that attached to the pterygoid bone, has begun to disappear, and the proximal end of the persisting distal portion lies alongside the distal end of the tensor veli palatini which has lost its attachment to Meckel's cartilage. This process continues, so that by stage J (fig. 28) the adjacent ends of the two muscles join end to end, separated only by a tendinous intersection. In stages C to F the proximal end of the tensor veli palatini is, as stated above, attached to the pterygoid bone; in stage H (fig. 26) it has additionally grown inwards below the pterygoid bone (in which a nodule of cartilage—the hamulus—has appeared) into the soft palate; and in stage J a well-marked transverse aponeurosis connects together the inner ends of the muscles of the two sides. A similar process occurs in Didelphys aurita.

In stage H there is a cap of dense tissue just above the dorsal end of the condylar process of the lower jaw, and into this the squamous bone is beginning to spread. In stage J the extension of the squamous inwards is more marked, and a split—the joint cavity—is developed in the dense tissue. Cartilage is developed in the condylar process in stage J (fig. 28). The external pterygoid is inserted into the condylar process and its neck in both stages.

In a 8·5 mm. embryo of Ornithorhynchus (figs. 29–33) the Anlagen of the incus, Meckel's cartilage, ala temporalis, and processus alaris are present in a precartilaginous condition. Aggregated mesoblast cells connect together the ala temporalis and processus alaris. The Anlage of the mandible is present, dorso-lateral to that of Meckel's cartilage. The

masticatory muscles consist of long striated muscle-cells, in two groups—a medial and lateral. The anterior fibres of the medial muscle descend vertically from just below the ala temporalis towards Meckel's cartilage, those next behind pass downwards and backwards, and the succeeding ones more and more backwards ; the hindmost reach as far back as the hind end of Meckel's cartilage ; there is no break in the muscle mass. The lateral muscle consists of fibres which, anteriorly, pass towards the upper edge and outer side of the anlage of the mandible ; the upper edge of the innermost fibres is close to the ala temporalis, whilst that of the more external fibres is outside the lateral surface of the Gasserian ganglion. The muscle-fibres which dorsally lie behind the hind end of the Anlage of the mandible pass downward and forwards towards its outer surface, and the lower end of this portion of the muscle is not continuous with that of the anterior portion ; dorsally, the two portions of the muscle are continuous.

The third division of the fifth nerve passes downwards between the medial and lateral muscles, giving off the lateral and medial rami to the muscles. The buccal nerve penetrates the inner fibres of the lateral muscle.

The intermandibularis forms a ventrally curved sheet, attached on each side to the inner surface of Meckel's cartilages. It does not at this stage extend further back. The depressor mandibulæ anterior is being proliferated transversely outwards from the under surface of the intermandibularis.

In a 25 mm. specimen of Echidna (= Stage 50 of Semon), the masticatory muscles consist of pterygo-tympanicus, tensor tympani, temporal, external pterygoid, zygomatico-mandibularis, masseter, and detrahens mandibulæ (figs. 34–36). The pterygo-tympanicus arises from the (Mammalian) pterygoid bone and is inserted into Meckel's cartilage. The tensor tympani arises from the (Mammalian) pterygoid bone and is inserted into the malleus portion of Meckel's cartilage. The ramus medialis of the mandibular division of the fifth nerve innervates these two muscles. The detrahens mandi-

bulæ arises from the outer end of the inturned crista parotica and is inserted into the outer surface of the mandible.

The intermandibularis (figs. 34–37) has spread behind the jaw as far back as the stylohyale, its anterior fibres—attached to the inner surface of Meckel's cartilage in stage 47—spread

FIG. 65.
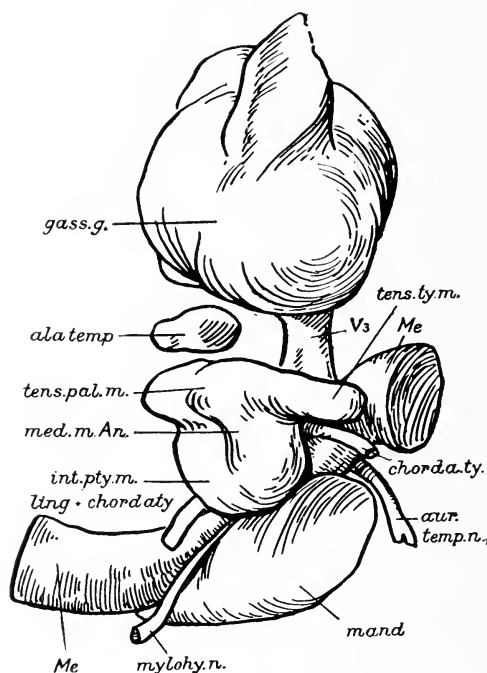
up towards the outer edge of the (Monotreme) pterygoid bone; the fibres next behind these are attached dorsally to the tympanic bone ; the most posterior fibres are not attached to the stylohyal. The depressor mandibulæ anterior is oblique in position, its inner end is more posterior than its outer, and is attached to dense connective tissue in front of the hyoid; the fibres pass outwards and forwards and are attached to the inner surface of the mandible.

In a 4½ mm. embryo of the rabbit the Anlagen of the masti-

catory muscles and the intermandibularis are continuous with
one another and lie internal to the third division of the fifth
nerve ; the upper limit of the cell column lies below the
Gasserian ganglion.

In a $5\frac{1}{4}$ mm. embryo (fig. 45) the Anlage of the masticatory

FIG. 66.
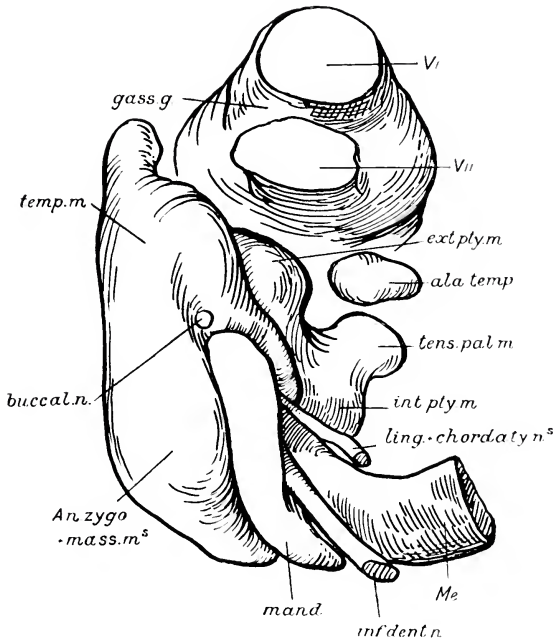
muscles spreads laterally in front of the third division of the
fifth nerve, so that the nerve passes down behind the Anlage ;
whilst the primitive position of nerve and muscle Anlage is
preserved lower down in the region of the intermandibularis.

In a 7 mm. embryo the Anlagen of the masticatory muscles
and intermandibularis separate from one another. The first
branch of the third division of the fifth nerve to be given off
is the buccal, which runs forward above the Anlage of the
masticatory muscles. The rami medialis and lateralis are
formed in an 8 mm. embryo, passing respectively inwards and

outwards into the masticatory Anlage. The ramus posterior is formed in a 9 mm. embryo.

In a 13 mm. rabbit embryo (figs. 65—67) the Gasserian ganglion is a relatively huge mass above the Anlagen of the masticatory muscles. Below the ganglion is the Anlage of the ala temporalis; the processus alaris is not yet formed.

FIG. 67.
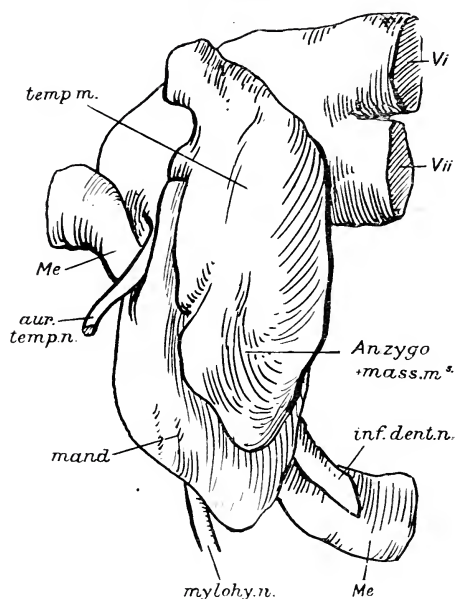
Meckel's cartilage is a slightly curved rod. The Anlage of the mandible, in which ossification has not yet begun, lies on the outside of Meckel's cartilage; its anterior edge does not extend quite so far forwards as that of the muscle-Anlagen; posteriorly its upper part ends abruptly except below, where there is a slight backward process. The auriculo-temporal nerve runs outwards and downwards behind the abrupt posterior edge and over the slight backward projection of the mandible. The Anlage of the masticatory muscles has not yet divided into medial and lateral portions, these being

continuous in front of the mandibular division of the fifth
nerve.   The medial portion extends downwards from just
below the ala temporalis inside Meckel's cartilage towards
the mandible; there is an inward bulge from its upper part
—the Anlage of the tensor veli palatini, and a long posterior
projection—the Anlage of the tensor tympani, extending
backwards to the malleus; the main mass of the medial por-
tion is the Anlage of the internal pterygoid.   The main mass

Fig. 68.
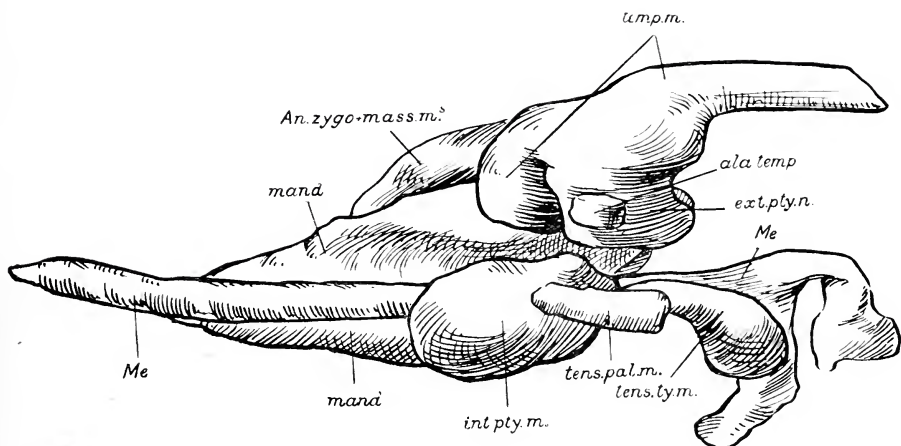
of the lateral portion (Anlage of temporal) has an upward
projection on the outer side of the Gasserian ganglion, its
lower end embraces the upper part of the mandible.   There
is a notch on its posterior border—the first indication of
the separation of a zygomatico-mandibularis and masseter
portion from the temporal, and an inward projection—the
Anlage of the external pterygoid, from the hinder part of its
inner aspect, towards the ala temporalis.

   In a 16 mm. embryo (figs. 46–48 and 68, 69) the ala orbitalis
and orbito-parietal commissure, the latter with a free posterior
extremity, are present.   The parietal-platte and processus
alaris are not formed.   The ala temporalis has no processus
ascendens.   The Anlage of the mandible has coronoid and

condylar processes. The Anlage of the masticatory muscles has divided into medial and lateral parts, each of which is imperfectly separated into muscles, which consist of groups of spindle-shaped cells. The medial part consists of the following: the internal pterygoid, which arises from the ala temporalis and is inserted into the mandible, it shows the beginning of a division into two parts. The tensor veli palatini lies just internal to the upper end of the internal

FIG. 69.

An zygo + mass. m.^{s}
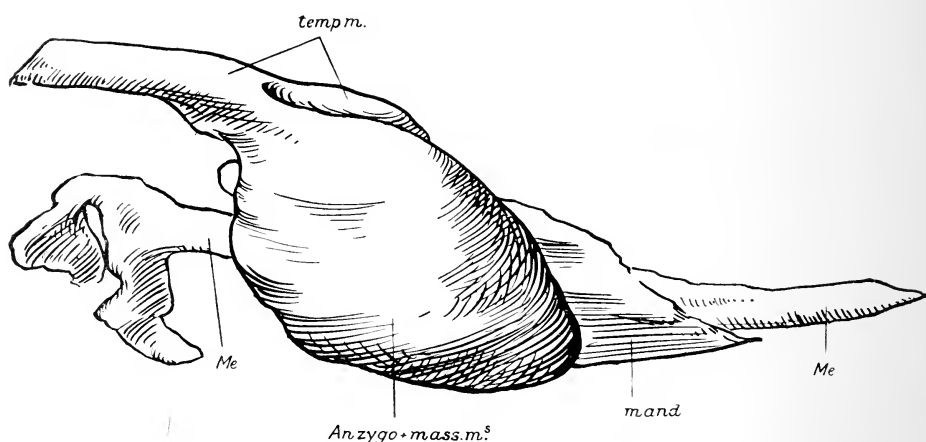
pterygoid with a free anterior end; it passes backwards above the hinder margin of the internal pterygoid and has a free posterior end. The tensor tympani lies immediately above and in contact with the hinder part of the tensor veli palatini, with a free anterior end; it passes backwards and inwards to the side of the malleus. The lateral part of the Anlage of the masticatory muscles consists of the following: the temporal, which has an oblique slightly concave inner edge; its upper extremity extends up outside the Gasserian ganglion, but has no dorsal attachment; it is inserted into the coronoid process. The external pterygoid arises from the ala temporalis behind the anterior fibres of the temporal and is inserted into the condylar process of the Anlage of the

mandible. The zygomatico-mandibularis-masseter is partially marked off from the external fork of the temporal by a groove passing upwards from the posterior edge.

In a 23 mm. embryo (figs. 49–53) the muscles have become quite separate from one another. The internal pterygoid now

Fig. 70.
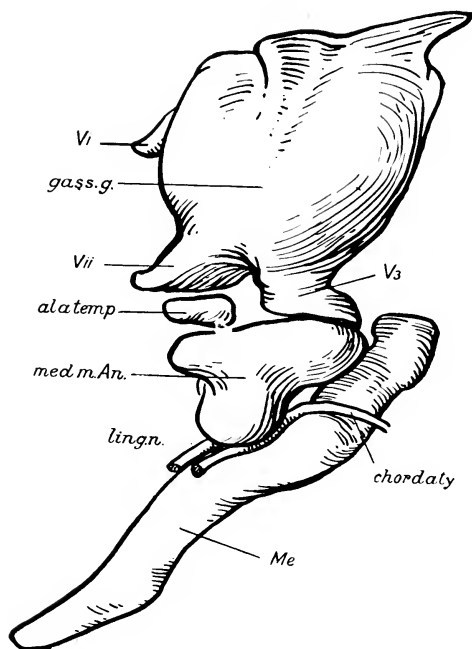
arises by two heads, one from the under-surface of the ala temporalis, the other from the palatine portion of the common Anlage of the palate and pterygoid bones; they unite and are inserted into the mandible. The anterior end of the tensor veli palatini winds round the hamulus into the soft palate; the muscle extends backwards medial to the internal pterygoid and is inserted partly to the tympanic bone, partly to the proximal end of the tensor tympani. The tensor tympani now lies wholly behind the internal pterygoid and tensor veli palatini owing to disappearance of its anterior

portion; it arises partly from the tendon of the tensor veli palatini, partly from the outer surface of the auditory capsule. The external pterygoid arises partly from the external surface of the ala temporalis and partly from the palatal portion of the common Anlage of the palate and pterygoid bones. The temporal arises by two heads, from the lamina

FIG. 71.
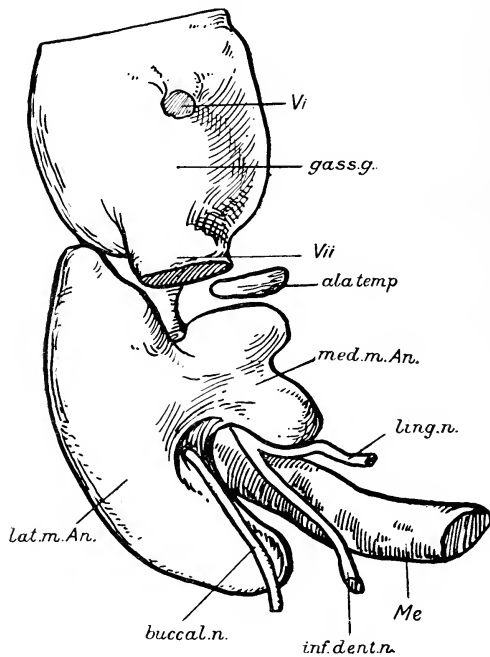
ascendens alæ temporalis and from the Anlage of the parietal bone. The masseter and zygomatico-mandibularis, not yet separated, are continuous dorsally with the temporal.

In a 33 mm. embryo (fig. 54) the zygomatico-mandibularis and masseter have become separated. The palatine and pterygoid bones have ossified, and one head of the external pterygoid arises from the palate bone.

Figs. 70 and 71 represent a model made from sections passing through the Anlage of the masticatory muscles in a

19 mm. embryonic pig ; the stage of development is a little less advanced than a 13 mm. rabbit embryo. Meckel's cartilage is a slightly curved rod of pre-cartilage ; its hinder portion and the Anlage of the mandible, though present, had

Fig. 72.
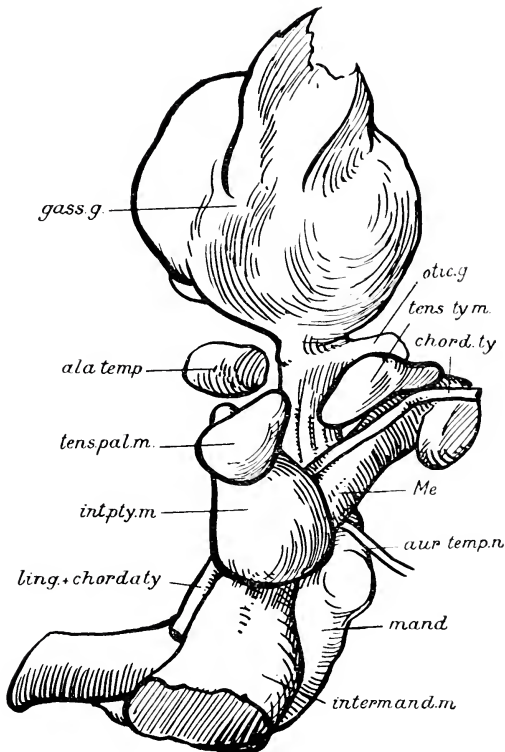
not sufficiently clear outlines to be modelled. The Anlage of the ala temporalis is present ; those of the ala orbitalis, orbito-parietal commissure, parietal-platte, and processus alaris, are not yet formed. The Anlage of the masticatory muscles forms a continuous mass, approximately ∧-shaped, with an apex just beneath the ala temporalis, and having an upward projection on the outer side of the Gasserian ganglion. The

outer limb of the mass, with the upward projection, is the
Anlage of the temporal, external pterygoid and masseter
muscles. The inner limb is the Anlage of the internal
pterygoid, tensor veli palatini, and tensor tympani; there is

FIG. 73.
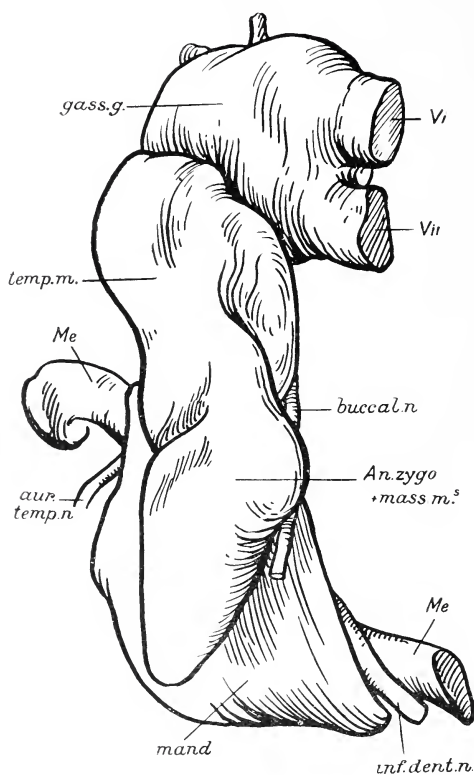
an inward bulge of its upper part—the future tensor veli
palatini, and a backward projecting part—the future tensor
tympani; its lower end is separated from Meckel's cartilage
by the chorda tympani.

The third division of the fifth nerve gives off the buccal
nerve (which penetrates the upper part of the outer limb of
the mass), then passes down behind the apex of the mass,

giving off the rami medialis and lateralis, and then forwards in the gap between the two limbs, dividing into auriculo-temporal, mylohyoid, inferior dental, and lingual branches.

Figs. 59–63 and 72, 73 are made from a 21 mm. pig embryo. The stage of development of the masticatory muscles is slightly more advanced than that of a 16 mm. rabbit embryo.   The ala orbitalis is continuous posteriorly with the orbito-parietal commissure, which does not reach the parietal-platte.   The ala temporalis is just beginning to chondrify.   The processus alaris is not yet formed.   The Anlage of the mandible, in which ossification has not begun, lies outside Meckel's cartilage and shows slightly marked coronoid and condylar processes. The Anlage of the masticatory muscles has divided into medial and lateral portions.   The former consists of internal pterygoid, tensor veli palatini and tensor tympani—which are separate structures, though very close together.   The internal pterygoid arises from the ala temporalis and is inserted into the mandible, the spindle-shaped cells not having any attachment to Meckel's cartilage.   The tensor veli palatini, which is not attached to any skeletal structure, lies internal to the upper end of the internal pterygoid, with its muscle-cells at right angles to those of the internal pterygoid.   The tensor tympani lies behind the tensor veli palatini, with a free anterior extremity; it is inserted, posteriorly, into the non-chondrified malleus portion of Meckel's cartilage.   The lateral portion of the Anlage of the masticatory muscles consists of temporal external pterygoid, zygomatico-mandibularis, and masseter muscles, which are only partially separated from one another. The temporal has no upper attachment, its lower anterior fibres are in front of the external pterygoid, those above extend upwards outside the Gasserian ganglion; it is inserted into the coronoid process of the mandible.   The external pterygoid arises from the ala temporalis and is inserted into the condylar process of the mandible.

The Anlage of the zygomatico-mandibularis and masseter is continuous with the temporal and is inserted into the Anlage of the mandible.   The united lingual and inferior dental

nerves pass between the internal and external pterygoids, then separate; the lingual is joined by the chorda tympani medial to the internal pterygoid and passes to the inferior maxillary ganglion; the inferior dental passes forward on the medial surface of the Anlage of the mandible. The mylohyoid branch of the inferior dental passes downwards between Meckel's cartilage and the Anlage of the mandible and then forwards on the lateral surface of the intermandibularis muscle. The auriculo-temporal passes outwards between Meckel's cartilage and the Anlage of the mandible.

In a 24 mm. pig embryo the condyloid and coronoid processes of the mandible are better marked, and ossification has begun. The upper end of the temporal has grown further upwards outside the membranous cranium, but is not attached to the orbito-parietal commissure. The Anlage of the parietal bone is not yet formed. The Anlage of the zygomatico-mandibularis and masseter arises partly from the temporal, partly from the Anlage of the zygomatic arch.

In a 32 mm. embryo (fig. 64), the orbito-parietal commissure is complete; it is covered by the Anlage of the parietal bone. The alisphenoid bone is not yet formed. The temporal arises from the parietal bone and the lamina ascendens alæ temporalis which is now formed. The Anlage of the zygomatico-mandibularis and masseter is beginning to separate into those two muscles. The internal pterygoid remains single, arising from the under surface of the ala temporalis. The proximal end of the tensor veli palatini has spread into the soft palate round the hamulus; its distal end is connected with the tensor tympani by a tendinous intersection.

The above-described phenomena show that the Anlage of the masticatory muscles in Mammals divides into medial and lateral portions, and that each develops into certain muscles. These may be taken separately.

Medial Muscle.—The primitive form of the medial muscle is present in 8·5 mm. embryos of Ornithorhynchus and stages A and B of Dasyurus, taking origin from the ala temporalis and inserted into the whole length of Meckel's

cartilage. This is not present in the rabbit and pig, being passed over in the Anlage stage.

The medial muscle or muscle-Anlage in Dasyurus, rabbit and pig, separates into three—from before backward, the internal pterygoid, tensor veli palatini, and tensor tympani. The internal pterygoid is the first to separate, the division into tensor veli palatini and tensor tympani occurring later. The internal pterygoid of Dasyurus first forms a muscle passing from the ala temporalis to Meckel's cartilage. On the occurrence of ossification it passes from the alisphenoid bone to the mandible and also gains an additional origin, from the palate and pterygoid bones. This is also the case in Didelphys aurita. In the rabbit and pig, the muscle at first arises from the ala temporalis and is inserted into the mandible—the stage of insertion into Meckel's cartilage being passed over. On ossification the muscle arises from the alisphenoid bone, with—in the case of the rabbit—an additional head from the palate bone.

In adult forms of Ornithorhynchus and Echidna there exists a muscle which was stated by Meckel and by Toldt to be homologous with the internal pterygoid of other mammals. Schulman stated that it is a true member—the caput anterius —of the temporalis group, as shown by its connection with the rest of the muscle and by its innervation by the N. temporalis profundus. This latter statement is confirmed by the evidence of embryos of Echidna in Stages 47 and 50 (of Semon); in these there is no muscle arising from the ala temporalis or (Mammalian) pterygoid bone and inserted into Meckel's cartilage or mandible. An internal pterygoid is thus absent.

An 8·5 mm. embryo of Ornithorhynchus (fig. 30) shows that the anterior fibres of the median masticatory muscle descend vertically to Meckel's cartilage, i. e. fibres are present homologous with those which form the internal pterygoid in Dasyurus. It may be concluded that this muscle atrophies in Monotremes, though whether its development in Ornithorhynchus advances a stage further than that just mentioned

before it atrophies could not be determined owing to want of material.

The pterygo-tympanicus s. tensor veli palatini is the middle one of the three into which the primitive medial muscle or muscle-Anlage separates.

Its development could not be followed in Ornitho-rhynchus. In Echidna—stage 50, the earliest available—the pterygo-tympanicus arises from the (Mammalian) pterygoid bone and is inserted into Meckel's cartilage. The muscle subsequently disappears, being absent in the adult (Schulmann).

In Dasyurus the pterygo-tympanicus arises from the pterygoid bone and is inserted into Meckel's cartilage, there being no stage in which, as a separate muscle, it arises from the ala temporalis. In later stages the distal end of the muscle loses its attachment to Meckel's cartilage and becomes connected with the persisting distal end of the tensor tympani. Insertion into the tympanic bone does not occur. At the same time the proximal end of the muscle—hitherto attached to the pterygoid bone—additionally grows inwards in the soft palate and forms an aponeurosis with the muscle of the opposite side. The pterygo-tympanicus thus becomes the tensor veli palatini. The same series of events occurs in Didelphys aurita.

In the rabbit the pterygo-tympanicus s. tensor veli palatini, when first visible as a separate muscle, lies on the medial side of the internal pterygoid. Its proximal end does not gain any attachment to the ala temporalis or pterygoid bone, but, later, grows inward in the soft palate round the hamulus. Its distal end becomes attached to the tympanic bone, but there is no antecedent stage comparable to that of Echidna and Dasyurus in which it is inserted into Meckel's cartilage. It also becomes attached to the persisting distal end of the tensor tympani. In the pig the process of development is abbreviated. The muscle, as in the rabbit, is first visible on the medial side of the internal pterygoid; its proximal end does not gain any attachment to either the ala temporalis or

pterygoid bone, but grows round the hamulus into the soft palate ; its distal end does not gain any insertion to either Meckel's cartilage or the tympanic bone, but becomes attached to the tensor tympani by tendon.   In Dasyurus, Didelphys, rabbit and pig, the development of the hamulus is synchronous with the ingrowth of the muscle into the soft palate.

On comparison of the descriptions of the pterygo-tympanicus given by Kostanecki, Schulman and Lubosch, those of the tensor veli palatini given by Kostanecki, and the embryological phenomena described above, it may be concluded that the muscle is derived from one taking origin from the ala temporalis and inserted into Meckel's cartilage.   This stage is not present in any of the animals investigated, but its existence, in phylogeny, may be inferred from the phenomena in early stages of Ornithorhynchus and Dasyurus, i.e. an undivided medial muscle passing from the ala temporalis to Meckel's cartilage, and after separation of this into parts, the insertion of the pterygo-tympanicus, in Echidna and Dasyurus, into Meckel's cartilage.

On the occurrence of ossification the muscle extended from the (Mammalian) pterygoid bone to the tympanic bone.   In Dasyurus this stage is present as regards the origin of the muscle but insertion into the tympanic bone is passed over ; in the rabbit it is present as regards the insertion but passed over as regards the origin ; in the pig neither attachment occurs.

The Anlage of the tympanic bone in Dasyurus is formed as a straight rod ventro-lateral to the hinder part of Meckel's cartilage, with its anterior part overlapped by the Anlage of the mandible ; subsequently an outgrowth downwards and backwards occurs, originating just behind the hind edge of the mandible.   This method of formation is in harmony with the theory of van Kampen that the tympanic bone was primarily a covering bone for the hinder part of Meckel's cartilage, and only subsequently—on development of a squamoso-mandibular jaw joint—became a part of the wall of the tympanic cavity. He suggested that it was derived from the supra-angulare or

angulare of the lower jaw, which are present in Amphibia and Reptiles. This opinion was adopted by Gaupp, who homologised the tympanic bone with the angulare, and pointed out that the presence of a pterygo-tympanicus in some Mammals was an additional fact in favour of van Kampen's theory.

It is probable, then, that the muscle—on the occurrence of ossification—arose from the (Mammalian) pterygoid bone, and was inserted into the meckelian (perhaps the only) portion of a tympanic bone which formed a covering bone for the old jaw with an incudo-meckelian joint. On the development of the new squamoso-mandibular joint, the muscle, being inserted behind this point, lost its significance as regards any action on the jaw, and as far as present information goes, this condition is not preserved in any adult Mammal.

No young enough Edentate embryos have been investigated to ascertain whether, in ontogenetic development, the muscle is inserted into the meckelian portion of the tympanic bone, but an indication of at least its phylogenetic existence is given by the temporary insertion of the muscle in Dasyurus into Meckel's cartilage opposite this portion of the bone. In late embryos of Dasyurus novemcincta and Bradypus marmoratus the muscle is inserted into the later formed lower limb of the bone.

Insertion solely into the tympanic bone is preserved to the adult condition in Bradypus tridactylus; the muscle generally spreads to neighbouring structures. Thus Schulman described its insertion in Ornithorhynchus to the junction of the os sphenoideum and os petrosum, in Cholœpus to the ligamentum accessorium mediale and walls of the fissura squamoso-petro-tympanica, in Manis to the "Trommelhöhle," in Tamandua to the bone round the cleft through which the chorda tympani passes; and Kostanecki described the insertion in Dasyurus sexcinctus to the bulla tympanica and os sphenoides.

The origin of the pterygo-tympanicus from the pterygoid bone is, similarly, rarely preserved; in Edentates only, as far as present information goes, in Tamandua (Schulman),

Tolypeutes (Lubosch), Bradypus tridactylus (Kostanecki) Manis pentadactyla (F. H. E.), whereas in Cholœpus and Manis Javanica (Schulman), Dasypus sexcinctus (Kostanecki), Dasypus novemcincta and Bradypus marmoratus (F. H. E.) the muscle spreads into the soft palate, forming a tensor veli palatini.[1] This secondary ingrowth remains in connection with the original pterygo-tympanicus, and may or may not have fibres taking origin from the pterygoid bone.

The condition present in the latter four Edentates hardly differs—as far as regards the proximal portion of the muscle —from that present in higher Mammals where the whole muscle, i. e. pterygo-tympanicus + its ingrowth into the soft palate, has generally been called the tensor veli palatini.

Schulman was of opinion that the relations of the pterygo-spinosus s. tympanicus of Ornithorhynchus indicated the existence of a movable (Monotreme) pterygoid in Pro-mammalia. In Echidna, however, the pterygo-tympanicus arises from the (Mammalian) pterygoid in stage 50 (though subsequently atrophying)—an occurrence that suggests that the origin of the muscle in the adult Ornithorhynchus from the (Monotreme) pterygoid is a secondary occurrence. Further, adoption of van Kampen's theory that the tympanic bone was, phylogenetically, a covering bone for a jaw with an incudo-meckelian joint, and so a movable structure, makes the theory improbable.

Lubosch doubted the homology of the pterygoid bone in the Zenarthra and Pholidota with that of other Mammals, and suggested that it might be homologous with the Echidna pterygoid, or possibly the result of fusion of a Mammalian with an Echidna pterygoid. The early stages are not yet known, but in Dasypus novemcincta (embryo 30 mm.) and Bradypus marmoratus (embryo 30 mm.) the relations of the pterygo-tympanicus muscle to that bone were identical with those in Dasyurus, and the bone, which showed no

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[1] Schulman was the first to recognise the genetic relationship between the pterygo-tympanicus and the tensor veli palatini.

evidence of fusion, lay ventro-median and close to the ala temporalis, and was apparently homologous with the ordinary Mammalian pterygoid.

The attachment of the (original) proximal[1] end of the tensor veli palatini to the pterygoid bone and hamulus is preserved in Man, Primates, Pinnipedia, Insectivora, Perisso-dactyla, Artiodactyla, Marsupials ; or it may altogether fail as in Cheiroptera, Carnivora, and Rodents (Kostanecki). The embryological phenomena in the rabbit and pig show that the latter occurrence may, at any rate in some cases, be due to its non-development.

The distal end of the tensor veli palatini in the rabbit becomes attached to the tympanic bone, whereas in Dasyurus, Didelphys and pig this ancient attachment is never gained. In the rabbit it persists up to the stage of 33 mm. (the oldest investigated), but judging from the account given by Kostanecki it is not present in any adult Mammal. It has yet to be determined in what orders other than Rodents a temporary attachment to the tympanic bone is gained. A secondary attachment to other adjacent bones is gained in some orders, e. g. to the bulla ossea in Marsupials and Rodents.

One almost constant feature of the distal end of the tensor veli palatini is attachment to the tensor tympani. Thus Kostanecki states that " Der Zusammenhang zwischen dem Tensor veli und Tensor tympani ist bei vielen Säugethieren erhalten, wo aber ein solcher verloren gegangen ist, weisen doch manche Punkte auf die früher bestehenden Beziehungen hin, die so sogar nur im Anschluss daran erklären lassen." The developmental phenomena in Dasyurus, pig and rabbit, show that this connection with the tensor tympani is intimately related to the atrophy or want of development of the proximal portion of the latter muscle.

[1] The nomenclature adopted here is in reference to the original form and attachments of the pterygo-tympanicus, from which the tensor veli palatini is derived, and is the reverse of that used by Kostanecki. The tensor veli palatini is an interesting instance of transference of " origin " from the proximal end to the distal end of a muscle.

The distal end of the tensor veli palatini also gains attach-
ment to the walls of the Eustachian tube, with which it is from
the very first in close relationship.

As regards innervation, Schulman determined that the
pterygo-spinosus of Ornithorhynchus is innervated by a
branch of the ramus medialis, s. ventralis, of the mandibular
division of the fifth. Lubosch did not describe the innerva-
tion of the pterygo-tympanicus of Edentates; but in Echidna,
Dasypus novemcincta, Bradypus marmoratus, and
Manis pentadactyla, I found the same innervation as in
Ornithorhynchus.

The tensor veli palatini has long been recognised as being
innervated by the ramus medialis of the mandibular division
of the fifth. I have recently confirmed this by showing that
in Macacus cynomolgus the muscle degenerates after
section of the fifth nerve proximal to the Gasserian
ganglion.

Tensor Tympani.—The tensor tympani in early stages of
Dasyurus passes from the pterygoid bone to the side of the
body of the malleus portion of Meckel's cartilage. Later on
the proximal portion of the muscle disappears, and the proximal
end of the persisting distal portion becomes attached to the
distal end of the tensor veli palatini and the cartilaginous wall
of the auditory capsule. It may be inferred that a stage has
been passed over in which the tensor tympani—as a separate
muscle—arose from the ala temporalis. Such a stage is
present in a 30 mm., i.e. relatively late embryo of Dasypus
novemcincta.

In Echidna, in stage 50, the tensor tympani passes from
the (Mammalian) pterygoid bone to the malleus. The proximal
portion of the muscle subsequently disappears, the proximal
end of the persisting distal portion being attached in the adult
to the petrous bone (Eschweiler).

In the rabbit the muscle never has any origin from either
the ala temporalis or the pterygoid bone, though a subsequently
atrophying, proximal portion of the muscle, overlapping the
tensor veli palatini, is formed. The proximal end of the

persisting distal portion becomes attached to the tensor veli palatini by tendon.

In the pig such a proximal portion is not formed; only the persisting distal portion, behind the Anlage of the tensor veli palatini, is developed, as found by Eschweiler.   All trace is thus lost, even in developmental stages, of the ancient origin from the pterygoid bone.

It may be concluded, from the descriptions given by Zuckerkandl, Kostanecki, Schulman, and Eschweiler, that the tensor tympani does not arise from the (Mammalian) pterygoid bone in any adult mammal.   This may be due to loss of such attachment during development, e. g. in Echidna, Dasyurus, Bradypus marmoratus, or to its non-formation, e. g. rabbit and pig.

Disappearance of the proximal portion of the muscle either by ontogenetic atrophy or by non-formation is a marked characteristic of the muscle.   In the former case it is apparently brought about by the bulging wall of the pars cochlearis of the auditory capsule interrupting the line of action of the muscle. The proximal end of the persisting, or solely developed, distal portion of the muscle gains secondary attachments, and especially to the pars cochlearis of the auditory capsule and Eustachian tube.   Of these attachments that to the Eustachian tube is in close association with the secondary insertion of the tensor veli palatini, as described above.

Eschweiler was of the opinion that these various attachments of the tensor tympani are derived from the condition found in Ornithorhynchus, where the muscle in the adult consists of two parts, both inserted into the malleus—a "Rachenbauch" which takes origin from "der Muskulatur hervor welche am hintern lateralen Choanenwand entspringt," and a "Felsenbeinbauch" springing from the labyrinth wall.

The development of the muscle in Ornithorhychus is not yet known, but comparison with Echidna, Dasyurus, pig and rabbit leads to the conclusion that in all probability the "Rachenbauch" of Ornithorhynchus is a secondary formation.

Temporal.—In Dasyurus the first origin of the lateral masticatory muscle (from which the temporal, masseter, zygomatico-mandibularis and external pterygoid are subsequently formed) is from the orbito-parietal commissure; the dorsal attachment of the muscle then spreads backwards to the parietal-platte and downwards to the membrana obturatoria covering in the spheno-parietal foramen, with its front edge to the lamina ascendens of the ala temporalis. On the occurrence of ossification the temporal muscle, which has been formed owing to the differentiation of the lateral group muscles, arises from the parietal and alisphenoid bones, the former being developed over the orbito-parietal commissure and parietal-platte, the latter over the ala temporalis, lamina ascendens and the membrana obturatoria.

In the rabbit and pig, with a longer intra-uterine development, the Anlage of the lateral masticatory muscle separates into the muscles it forms before attachment takes place. The temporal becomes attached to the lamina ascendens alæ temporalis, and spreads upwards to the territory of the orbito-parietal commissure and parietal-platte, but these latter structures are by that time covered by the Anlage of the parietal bone from which the muscle gains an origin. On the subsequent ossification of the alisphenoid bone the lower fibres arise from that bone.

The lamina ascendens alæ temporalis is complete in Dasyurus and Didelphys aurita; it has a free upper end in rabbit, pig, and Dasypus. The orbito-parietal commissure is complete in all four animals.

The lower end of the lateral masticatory muscle in the just born Dasyurus is at first attached to the upper edge of Meckel's cartilage viâ a mass of cells in which the Anlage of the mandible is just visible. Ossification subsequently extends upwards forming a coronoid process and downwards on the lateral side of Meckel's cartilage; in relation with these changes the insertion of the muscle becomes forked and laps the coronoid process on both sides, though to a greater extent laterally than medially.

In the rabbit and pig there is no stage in which the temporal muscle is inserted solely into the upper edge of the Anlage of the mandible; its cells become spindle-shaped before it spreads downwards, with a forked extremity, on either side of the Anlage of the mandible.

The formation of the coronoid process of the mandible takes place in Dasyurus after ossification has begun, in the rabbit and pig in the Anlage stage. In all three animals the process is formed by a dorsal extension into an already formed muscle or muscle-Anlage.

The stages of Ornithorhynchus and Echidna available did not permit of observations on the development of the individual muscles formed from the lateral masticatory muscle.

Masseter and Zygomatico-mandibularis.—In many Mammals a zygomatico-mandibularis is differentiated between the temporal and masseter muscles. Allen, who first described the muscle, in man, considered it to be a separated deep portion of the masseter, whilst Toldt held that it is derived from the temporal. Toldt stated that " er stammt von der Muskelfasergruppe her, welche die oberflächlichste Schichte der ursprünglichen Anlage des Schläfenmuskels darstellt und von jener Mesodermschichte kommt welche sich bleibend zur Fascia temporalis gestaltet. Da sich in derselben Mesoderm-schichte später das Jochbein, beziehungsweise der ganze Jochbogen entwickelt, so tritt ein bestimmter Anteil der bezeichneten Fasergruppe mit der medialen Fläche des Jochbogens in Beziehung, d. h. er nimmt von dieser bis an ihren unteren Rand herab bleibend seinen Ursprung. Er steht demgemäss an seinen Ursprung mit dem Schläfenmuskel in ununterbrochenen Zusammenhang, hat aber durch die erworbene Beziehung zum Jochbogen einen besonderen Character erlangt."

In Dasyurus, as detailed above, the masseter muscle is proliferated, in stage C, downwards and backwards from the anterior ventral edge of the lateral muscle on the outside of the mandible; and from that time onward can be distinguished in transverse section by the direction of its fibres. The

zygomatico-mandibularis is formed subsequently, in stage H, by a separation of the ventral fibres of the temporal muscle, dorso-posterior to the masseter muscle. The fibres of the zygomatico-mandibularis have the same direction as those of the temporal, and up to stage J—the latest investigated—the separation was not complete.

In the rabbit and pig the process is a little different. The Anlage of the lateral masticatory muscle is inserted by a forked lower edge into the mandible. A groove appears in the posterior edge of the external fork, and spreading upwards and forwards separates the common Anlage of the masseter and zygomatico-mandibularis from the temporal. This Anlage divides into masseter and zygomatico-mandibularis, the latter being cut off from the upper posterior part of the common mass. In view of the many modifications associated with the prolonged intra-uterine development, it is probable that the phenomena occurring in Dasyurus more closely represent the phylogenetic history of the muscles than do those of the rabbit and pig. Both masseter and zygomatico-mandibularis have their first insertion into the Anlage of the mandible. Neither has any temporary insertion into Meckel's cartilage.

The detrahens mandibulæ is present only in Monotremes. No trace of it was seen in the developmental stages of Dasyurus, pig and rabbit. It is innervated by the fifth nerve (Westling, Fürbringer, Gaupp, Schulman). Schulman regarded it as belonging to the dorsal (in this paper regarded as " lateral ") group of masticatory muscles as—like the temporal, external pterygoid, masseter, and zygomatico-mandibularis—he found it to be supplied by the dorsal s. lateral division of the mandibular nerve.

Owing to want of material its development could not be completely followed in either Ornithorhynchus or Echidna, but the stages described above permit of an approximate account. The muscle can be identified with the hinder fibres of the lateral masticatory muscle in 8·5 mm. embryos of Ornithorhynchus—those fibres which pass downwards and forwards towards the outer surface of the hind end of the Anlage of

the mandible. In stage 47 of Echidna the muscle has separated off and passes from the root of the crista parotica downwards and forwards to the outer surface of the mandible. This condition is also present in stage 50. In the adult Echidna this primary origin is lost, and the muscle arises from the ventral surface of the squamosal bone (Schulman).

These phenomena support the view of Schulman that the detrahens mandibulæ is one of the lateral group of masticatory muscles. They negative the view of Toldt that it is split off from an already differentiated masseter, and suggest that the muscle is, phylogenetically, more ancient than either the masseter, zygomatico-mandibularis or external pterygoid.

The external pterygoid muscle is developed from the temporal in Dasyurus, pig and rabbit. Its origin in Ornithorhynchus and Echidna was not determined owing to a want of the necessary stages. The process could be most clearly seen in Dasyurus, where the cells forming the muscle are at first small and non-striated, and probably proliferated from, and not formed by change of direction of, the longer cross-striated muscle cells of the temporal. In the rabbit and pig the formation of the external pterygoid takes place in an Anlage consisting of oval cells, and appeared to be due to separation of a portion of the temporal Anlage.

The primary origin of the external pterygoid in Echidna is from the membrana obturatoria above the ala temporalis; in Dasyurus, and Didelphys aurita it is from the lower end of the lamina ascendens alæ temporalis; in the rabbit and pig from the ala temporalis. On the formation of the alisphenoid bone outside the ala and its lamina ascendens, the muscle—in Dasyurus, Didelphys, rabbit and pig—arises from this bone; and in the rabbit it gains an additional origin from the palate bone. In the adult Echidna (Schulman) the muscle arises from the planum infratemporale of the great wing of the sphenoid and from the ala temporalis palatini.

Origin of the muscle from the alisphenoid bone is common: thus Lubosch describes the origin of the muscle, in Bradypus from the pterygoid bone, in Dasypus and Tolypeutes by

two heads from the alisphenoid, in Tatusia by two heads, one from the alisphenoid, the other from the alisphenoid and palate bones, in Tamandua by two heads, one from the parietal, the other from the palate.

The first insertion of the external pterygoid is into the condylar process of either the ossified mandible (Dasyurus) or its Anlage (rabbit and pig). There is no transitory insertion into Meckel's cartilage. The development of a condylar process is synchronous with the development of the external pterygoid. These phenomena support the view of Gaupp that the condylar process is primarily a " Muskelfortsatz."

An external pterygoid muscle is present in most Mammals. Two exceptions have been recorded and are of considerable interest. Leche was of opinion that the internal and external pterygoid muscles " auf einen gemeinsamen Ursprung zurück-gefuhrt werden können," but did not record any instances other than that of Phoca, in which one muscle only was present, which might represent a common muscle mass from which in other Mammals internal and external pterygoids were differentiated.

Humphry had described in Phoca communis one ptery-goid muscle only " arising from the outer side and edge of the slightly developed pterygoid part of the sphenoid and passing to the inner side of the angular part of the jaw." He did not mention its innervation nor did he describe the temporal muscle.

I find that in Halichœrus grypus (grey seal) the masseter, zygomatico-mandibularis, temporal, external pterygoid, internal pterygoid, tensor veli palatini and tensor tympani muscles are present. The temporal is inserted into the coronoid process, the external pterygoid into the condyloid process and interarticular meniscus, and the internal pterygoid into the inner side of the angular part of the jaw. The ramus lateralis of the mandibular division of the trigeminal nerve supplies the masseter, zygomatico-mandibularis, temporal (by three twigs), and the external pterygoid ; the ramus medialis supplies the internal pterygoid, tensor veli palatini, and tensor tympani.

In Phoca communis s. vitulina (common seal) there is one pterygoid muscle only, as described by Humphry; it agrees in form, origin, insertion and innervation from the ramus medialis, with the internal pterygoid of Halichœrus. The "temporal" is inserted into the interarticular meniscus, the neck of the condyloid process and the coronoid process (fig. 44); it is innervated by four twigs from the ramus lateralis.

It is clear that the "temporal" of Phoca represents the temporal + external pterygoid of Halichœrus, and the condition is probably due to non-separation of these two muscles —a persistence of an embryonic stage.

The matter, however, is complicated by the statement of Toldt that in Phoca vitulina both pterygoid muscles exist— a statement which does not agree with the findings of Humphry and myself in Phoca vitulina, though it does agree with what I found in Halichœrus grypus.

In Manis tetradactyla, according to Lubosch, there is only one pterygoid muscle arising from the palate bone and passing down internal to the N. mandibularis. No separate external pterygoid was found. Lubosch was of the opinion that " er mit dem inneren Flügelmuskel verschmolzen ist, und ich beziehe die occipitalsten Fasern seiner Ansatzes, die in der Figur an den Condylus tretend gezeichnet sind, auf ihn." I found a similar condition in Manis pentadactyla.

The above-described observations show that the Anlage of the masticatory muscles in Mammals divides into medial and lateral portions. From the medial portion or muscle are developed the internal pterygoid, pterygo-tympanicus or tensor veli palatini, and the tensor tympani. From the lateral portion or muscle are developed the temporal, masseter, zygomatico-mandibularis, and external pterygoid.

These phenomena of development are in harmony with, and offer an explanation of, the method of innervation by the mandibular division of the fifth nerve. The muscles developed from the medial portion are innervated by the ramus medialis, those developed from the lateral portion by the ramus lateralis.

The division of the muscles and nerve-branches into medial and lateral groups is further evidenced, as I have recently shown, by the differing paths of the nerve-fibres—both motor and sensory—into the rami.   The fibres of the ramus lateralis have a simple direct path, whilst the fibres of the ramus medialis are for a space split into two parts by the ramus posterior.[1]   Both rami contain motor fibres from the motor root, and muscle-sensory fibres from the Gasserian ganglion.

The division is also corrobated by the grouping of the cells in the motor nucleus of the trigeminal nerve.   Willems found three groups of cells in this nucleus[2] in the rabbit—a dorsal, a ventro-median, and a ventro-lateral.   The occurrence of chromolytic changes after avulsion of individual motor branches showed that the external pterygoid, the temporal and sphenoidal,[3] and the masseter, are innervated by the dorsal group, the internal pterygoid by the ventro-lateral group, the mylohyoid and the anterior digastric by the ventro-median group.   No chromolytic changes were found after avulsion of the tensor tympani, and no operations were performed on the tensor veli palatini.

Willems was of the opinion that "ces groupements cel-lulaires répondent relativement bien à des functions différ-entes."   The internal pterygoid, however, is more closely associated in function with the temporal and masseter than is the external pterygoid.   The grouping agrees with the division of their Anlage into lateral and medial masticatory muscles or muscle-Anlagen during development, and is a morphological one—probably dating from a period antecedent

[1] I showed this to be the case in Man, Macacus, dog, and rabbit.   I can now also add Dasyurus, Ornithorhynchus, and Echidna.

[2] Willems applied the term "masticatory nucleus" to the whole of the motor nucleus of the trigeminus.   In this paper the term "masticatory" is used—as is usual in English text-books—to denote those muscles innervated by the fifth cranial nerve which are situated dorsal to the lower jaw, i. e. to the exclusion of the mylohyoid and anterior digastric, with a similar restriction in the case of the parts of the motor nucleus.

[3] The part of the temporal innervated by the anterior deep temporal nerve.

to the differentiation of the individual muscles which characterise Mammals. Similarly, the ventro-median group probably dates from a period antecedent to the differentiation of a depressor mandibulæ anterior s. anterior digastric from the intermandibularis.

The buccal nerve is generally described as a branch of the ramus lateralis, alongside of which it runs for a short distance in the adult. It would be preferable, however, in view of its earlier development and different function, to describe it as a separate branch of the mandibular division of the fifth nerve, and to restrict the term " ramus lateralis " to the muscular branch.

The intermandibularis in early stages of Ornitho-rhynchus, Dasyurus, rabbit and pig forms a ventrally curved muscular sheet, with a median raphé, and attached laterally to the inner surface of Meckel's cartilage. At a late stage, in Dasyurus, pig and rabbit, it becomes attached to the mandible. In Ornithorhynchus (Schulman) the muscle is attached laterally to the lower jaw, ligamentum pterygo-mandibulare, os pterygoideum and annulus tympanicus, the last-mentioned portion forming a separate muscle, the tympanico-hyoideus. In Echidna, stage 47, the muscle has already extended backward, but its anterior part is still attached to Meckel's cartilage ; in stage 50 this attachment is lost and the fibres extend up towards the outer edge of the (Monotreme) pterygoid bone ; the fibres behind are attached to the tympanic bone. In the adult (Schulman) the muscle is attached to a fascial sheet at the level of the foramen rotundum, the hinder half of the outer edge of the os palatinum, the os pterygoideum, the ligamentum pterygo-mandibulare, the tympanicum and the stylohyal. The above mentioned embryo-logical phenomena support his view that the absence of attach-ment of the muscle to the jaw is a secondary occurrence.

On the Homologies between the Masticatory Muscles of Mammals and Non-mammals.—The embryo-logical evidence detailed above suggests that Mammals have originated from forms characterised by two masticatory

muscles only : a medial arising from the ala temporalis and inserted into Meckel's cartilage and innervated by the ramus medialis of the mandibular division of the fifth nerve, and a lateral arising from the orbito-parietal commissure and inserted into a rudimentary mandible and innervated by the ramus lateralis.

In Sauropsida[1] the Anlage of the masticatory muscles divides into upper and lower portions, the former inserted into the palatine process of the quadrate, the latter extending from the palatine process of the quadrate to Meckel's cartilage. The upper portion forms the spheno-pterygo-quadratus or a homologue of this muscle, or—as in Chelonia and Crocodilia— atrophies.   The lower portion of the Anlage of the mastica- tory muscles separates into inner and outer parts, the inner developing into the pterygoid muscle or muscles, and the outer into the capiti-mandibularis.   The dorsal end of the capiti-mandibularis, at first attached to the palato-quadrate, grows upwards and gains a dorsal attachment to the skull.

Thus, whilst in Mammals the Anlage of the masticatory muscles does not divide into upper and lower portions, in Sauropsida it does so.   This cardinal difference is not taken into account by Fürbringer, Kostanecki, Gaupp, and Cords, so that the various homologies which they regard as existing between individual masticatory muscles in these two great Vertebrate groups are questionable.

In Amphibia the Anlage of the masticatory muscle does not divide into upper and lower portions, above and below the palatine process of the quadrate.   The whole dorso-ventral strip separates into a medial and a lateral muscle.

The initial stages of development of the masticatory muscles in Mammals are thus exactly comparable to those of

[1] Only the barest outline of the phenomena is stated here.   In the 'Quarterly Journal of Microscopical Science,' vol. 51, 1907, I gave a short account, in all groups of the Sauropsida, with an analysis of the somewhat bewildering array of names given to the muscles by various observers.

Amphibia, and a distinct homology exists between the medial
and lateral masticatory muscles in the two Vertebrate groups.
In Mammals these two muscles undergo considerable changes
as detailed above, so that no homology exists between any
one Mammalian masticatory muscle and any one Amphibian
muscle.

The intermandibularis is homologous with the similarly
named muscle in other Vertebrate groups, Elasmobranchs,
Teleostomi, Amphibia and Sauropsida.   Its development in
mammals, just as in other Vertebrates, lends no support to the
theory of Ruge that it is primarily a hyoid muscle.

As will be shown later (pp. 579, 613, 616), the depressor
mandibularis anterior of Ornithorhynchus and the anterior
digastric of Dasyurus, rabbit and pig, are formed by pro-
liferation from the ventral surface of the intermandibularis.
There are no homologies in other Vertebrate groups, so that
the muscle must have arisen within the Mammalian phylum.

On the Changes in the Jaw Muscles accompany-
ing the Development of the Squamoso-mandibular
Joint in Mammals.—Dasyurus and Echidna are born at an
early stage with a functional incudo-meckelian joint, and the
change of jaw-joint occurs during the pouch stage of existence.

In the earlier stages of Dasyurus the prominent feature is
the great development of the lingual muscles—the genio-
glossus, hyo-glossus, stylo-glossus, and transverse lingual
muscle-fibres ; this and the concavity of the anterior part of
the dorsal surface of the tongue are intimately associated with
the intrabuccal position of the maternal teat.   Then follows the
development of two new muscles—the transversely directed
anterior digastric, which soon, however, becomes more oblique
in position, with its medial end attached to the transverse
aponeurosis of the hyoid ventral constrictor, and the external
pterygoid.   The adjacent ends of the genio-hyoid, sterno-
hyoid and omo-hyoid lose their attachment to the first branchial
cartilage and become united by tendon.   These muscle phe-
nomena precede and apparently initiate the development of
the squamoso-mandibular joint.   In Phascolarctos, however,

the genio-hyoid retains its attachment to the basibranchial, and in Didelphys aurita the hyoglossus is attached to the first branchial bar. In Echidna,[1] where there is no mammary teat, there is, in stage 50, a complicated system of protractors and retractors of the tongue, the former consisting of genio-glossus, genio-glossus posticus externus and internus, the latter of sterno-glossus and laryngo-glossus (figs. 34–37). The sterno-glossus passes through a loop formed by a M. annulus. The transverse lingual fibres are not specially developed. The external pterygoid and depressor mandibulæ anterior are developed. The posterior end of the genio-hyoid is attached both to the first branchial cornu and to the thyroid cartilage. A sterno-thyroid is attached anteriorly to the posterior edge of the thyroid cartilage. The omo-hyoid, arising from the medial surface of the scapula, is in part inserted into the thyroid cartilage and in part joins the genio-hyoid.

On comparison of the above phenomena it would appear that there are two common characteristics of the jaw-muscles in Marsupials and Monotremes which may be supposed to have played a part in the phylogenetic change of an incudo-meckelian to a squamoso-mandibular joint : (1) The development of an external pterygoid. (2) The development of a (transverse) depressor mandibulæ anterior. This muscle in Dasyurus quickly becomes oblique and forms the anterior digastric. The condition in Monotremes, however, negatives any idea that a digastric had any share in the phylogenetic development of a squamoso-mandibular joint. In Echidna and Marsupials various secondary connections are developed between the elements of the hypo-branchial spinal muscles which are situated behind the first branchial bar (i. e. sterno-hyoid, omo-hyoid, and thyro-hyoid) and those in front (i.e.

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[1] This account differs from that given by Fewkes in (1) the more extended posterior insertion of the genio-hyoid, (2) the existence of a sterno-thyroid, (3) the existence of an omo-hyoid—confirming the statement of Leche. The M. annulus was not yet separated into M. annulus inferior and M. annulus intimus.

genio-hyoid, stylo-glossus, and hyoglossus), whereby a series of long retractors of the front end of Meckel's cartilage and of the tongue are formed. Though such retractors may possibly play a part in the formation of the new jaw-joint in the individual, yet the absence of any uniformity in their development suggests that they did not do so in its phylogenetic development.

It has generally been supposed that the formation of the new squamoso-mandibular joint was associated with the disappearance of a depressor mandibulæ. As is stated later (p. 630), there is no ontogenetic evidence of the disappearance of such a muscle, and comparison of the early stages of the hyoid muscles of Amphibia and Sauropsida with those of Mammals shows that the levator hyoidei—from which in Amphibia and Sauropsida the depressor mandibulæ is derived—preserves in Mammals, and especially in Monotremes, its primary condition as a muscle affixed to the hyoid bar.

The above considerations support the theory of Fürbringer that the change of jaw-joint was associated with the development of the mammary function: "Dieses Abweichen der Mammalia von dem fortschreitenden Entwickelungsgange, wie ihn Amphibien und Sauropsiden einschlagen, legt den Gedanken nahe, dass bei ihnen in jugendlichen Entwickelungsstadien, in den Beuteljungenkindheit, ein den Säugethieren eigentümlicher äusserer Anstoss eintrat welcher zu dieser Lockerung des Unterkiefers Veranlassung gab. Ich neige dazu, diesen in der saugenden Tätigkeit der Beuteljungen zu erblicken. Wie bei den Anurenlarven nur der vordere Teil des zu einiger Selbständigkeit gestalteten Unterkiefers zum Anheften und Kauen verwendet wurde, so dient auch bei den mammalen Beuteljungen nur der vordere Abschnitt der Mandibula der Saugfunction, welche zusammen mit der Ausbildung der Milchdrüsen eine neue Erregungsschaft gegenüber allen Nicht-Säugetieren ist."

Some of the muscle-phenomena appear to be secondary to the change of jaw-joint. The pterygo-tympanicus of Dasyurus and higher Mammals becomes transformed into the tensor

veli palatini, though in Ornithorhynchus and some Edentates
its ancient insertion into the tympanic bone is still preserved.
The proximal end of the tensor tympani disappears and the
muscle gains a new origin. The disappearance, in the ontogeny
of Dasyurus, pig and rabbit of the detrahens mandibulæ may
also be related to the change of jaw-joint. Two slightly
different views as to this muscle have been put forward.
Toldt held that the muscle " bei der Neubildung des Kieferge-
lenkes, als ein infolge des Schwindens des M. depressor
mandibulæ notwendig gewordener Factor für die Kieferbe-
wegung, von dem M. masseter abgespalten hat"; whilst
Gaupp was of the opinion that it " sich zugleich mit dem
Masseter selbst aus einer gemeinsamen Muskelmasse der
Reptilien (dem sogenannten M. capiti-mandibularis) bei der
Neubildung des Kiefergelenkes herandifferenzierte." Ac-
cording to both theories the muscle was developed with the
new squamoso-mandibular joint. The above-recorded observa-
tions, however, suggest a converse explanation—that it is an
old muscle possessed by Mammalian ancestors with an incudo-
meckelian jaw-joint, which has dropped out, except in
Monotremes, owing to its being inserted behind the new joint.
The phylogenetic history would thus present an interesting
contrast to that of the pterygo-tympanicus and tensor
tympani.

The theory above advocated differs in some particulars
from that advanced by Gaupp. In his treatise on " Die
Reichertsche Theorie " it is assumed that the substitution of
an incudo-meckelian jaw-joint by a squamoso-mandibular one
was accompanied by a change in the masticatory muscles—
from a Reptilian to a Mammalian type. The developmental
phenomena occurring in the muscles, however, suggest that
this substitution occurred subsequent to changes in the
masticatory muscles from what may be called a pre-amphibian
type, i. e. one characterised by median and lateral masticatory
muscles and a levator hyoidei, to a Mammalian one.

The amount of such change is very doubtful. Possibly
Mammalian ancestors with an incudo-meckelian jaw-joint

possessed internal pterygoid, a muscle representing the pterygo-tympanicus and tensor tympani (derived from the medial muscle) ; temporal and detrahens mandibulæ (derived from the lateral muscle) ; but not external pterygoid, masseter, zygomatico-mandibularis, or depressor mandibulæ anterior.

Muscles of the Hyoid Segment.

In stage A of the Dasyurus (fig. 3) the hyoid apparatus consists of hyoid and first branchial bars and a basibranchial. There is no basihyal. The ventral ends of the hyoid and first branchial bars are continuous with the basi-branchial. The hyoid bar forms a continuous structure—of stapes, inter-hyale, and stylo-hyale; the latero-hyale extends upwards from the interhyale to the auditory capsule. There is as yet no crista parotica. The only chondrified parts of the hyoid bar are the extreme lower end and the middle part; to the latter are attached the stylo-pharyngeus on the inside and the upper end of the posterior digastric on the outside. The posterior end of the stylo-glossus is attached to the tendinous part of the hyoid bar just below the middle cartilaginous part. The branchio-hyoid passes from the first branchial to the hyoid bar. The adjacent ends of the genio-hyoid, omo-hyoid and sterno-hyoid are attached to the first branchial bar. The thyro-hyoid is not yet developed. There are two hyoid muscles—the stapedius and posterior digastric. The former arises from the outer chondrified wall of the upper part of the auditory capsule and passes downwards and forwards to be inserted into the back of the upper part of the stylo-hyale. The posterior digastric takes origin from the middle chondrified portion of the hyoid bar, and passes downward and forwards outside the bar to a transverse tendon connecting the muscle to its fellow just behind and slightly underlapping the inter-mandibularis. No trace of the anterior digastric or of the Anlage of the sphincter profundus and platysma is visible. The N. facialis passes backward on the external surface of the stapedius and then downwards on the external surface of the posterior digastric.

In stage C (figs. 7—10 and 12) the stapes[1] has chondrified. The crista parotica is beginning to form, as a slight downward extension from the chondrified portion of the outer wall of the auditory capsule. It is deepest in front, where the latero-hyale is attached, and gradually lessens behind. The stapedius muscle, taking origin from the lower edge of the crista parotica, passes forwards and downwards to the hyoid bar below the interhyale. The upper end of the posterior digastric—attached solely to the hyoid bar in stages A and B—has now an additional origin, by a long tendon passing upwards behind the hyoid bar to the paroccipital process.

In stage D (fig. 19) chondrification has extended from the middle portion of the hyoid bar into the latero-hyale, the pars inter-hyalis remaining precartilaginous. The origin of the stapedius muscle has shifted still further inwards, the muscle arising from the floor of the fossa stapedii; it is inserted into the hyoid bar below the inter-hyale.

In stage F the inter-hyale has disappeared, and the cartilaginous latero-hyale and stylo-hyale, but for a small nodule, are replaced by a ligament which, ventrally, is continuous with the cartilaginous hyoid cornu of the hyoid apparatus. The insertion of the stapedius has shifted inwards to the stapes.

In stage H the attachment of the posterior digastric to the hyoid bar is lost; its attachment to the paroccipital process is tendinous up to stage J—the latest investigated—but in the adult it has a fleshy origin.

The condition of the stapes, stapedius and posterior digastric muscles of 10 mm. specimens of Didelphys aurita is similar to that of the Dasyurus in stage H.

The anterior digastric is proliferated from the hinder part of the intermandibularis in stage D (fig. 18). The fibres are given off from the ventral surface of the posterior portion of the muscle and are directed transversely outwards, and have

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[1] A stapedial artery passes through the stapes in stages A–C; it disappears subsequently.

no lateral attachment. In stage E (fig. 21) the muscle has separated from the intermandibularis; its ventral (inner) end has become attached to the ventral aponeurosis of the posterior digastric.

In stage F (fig. 22) the muscle is more obliquely situated, the muscle-fibres passing from its posterior attachment to the posterior digastric forwards and outwards to the ventral edge of the mandible. A digastric muscle is thus formed.

In an 8·5 mm. embryo of Ornithorhynchus (figs. 30, 31, 33), the hyoid bar, in a pre-cartilaginous condition, is a continuous structure passing from the stapedial portion above to near the middle line below. The upper, stapedial end abuts against the auditory capsule, which consists of aggregated mesoblast cells. There is no crista parotica and no latero-hyale. The hyoid muscles consist of a levator hyoidei[1] and a hyoid ventral constrictor s. styloideus, which are not quite separated from one another. The levator hyoidei has no dorsal attachment; it is inserted into the hyoid bar. The N. facialis passes backward lateral to the muscle. The hyoid ventral constrictor arises from the hyoid bar and passes down to a ventral aponeurosis connecting together the muscles of the two sides.

The hyoid muscles of Echidna, in stage 50 of Semon, consist of a levator hyoidei and hyoid ventral constrictor s. styloideus (fig. 37). The levator hyoidei arises from the outer end of the inturned crista parotica and is inserted into the upper end of the stylo-hyal cartilage. The hyoid ventral constrictor arises from the stylo-hyal cartilage just below the insertion of the levator hyoidei and passes downwards to a median ventral aponeurosis.

The following is a summary of Futamura's observations (1907) on the development of the hyoid muscles in the pig. In a 8·4 mm. embryo their Anlage consists of an aggregation of myogenic cells which is continuous ventrally with its fellow across the middle line. It is penetrated by the seventh nerve.

[1] The introduction of this term is explained later; the muscle is homologous with the stapedius of non-Monotreme Mammals, but never gains any insertion into the stapes.

In a 11 mm. embryo the Anlage has split into a superficial and a deep layer—the former is the Anlage of the platysma, the latter that of the stapedius, digastricus, and stylo-hyoid. In a 15 mm. embryo the antero-posterior fibres of the stapedius form the most dorsal portion of the Anlage; immediately below these is the upper end of the digastricus, i. e. this muscle has not yet extended up to the mastoid process. The digastricus is in two parts, of which the posterior (innervated by the N. facialis) is inserted into Reichert's cartilage; the anterior passes round the cartilage and is inserted in Meckel's cartilage. A small outgrowth from the posterior part is the Anlage of the stylo-hyoid. In a 21 mm. embryo the upper end of the digastricus has spread up to the mastoid process, and the jugulo-hyoideus has separated from its upper part and is inserted into the hyoid bar. The stylo-hyoid is fully separated.

Kallius (1909) described the development of some of the hyoid muscles in his account of the tongue and associated structures of the pig. In stage 12 (= Keibel's stage 22) " von dem proximalen Ende des Knorpelanlage des zweiten Schundbogen zieht ein schmaler Strang von wenig differenzierten Muskelblastem nach der Mittellinie zu in die Gegend des oralen Endes der Copula des Branchialskeletes." There is no trace of an anterior digastric. In stage 17 (= between twenty-seventh and twenty-eighth stages of Keibel) the anterior digastric forms a well " abgrenzbaren Muskelbundel welcher offenbar mit der ersten Portion (Mylohyoideus) von der ursprünglich einheitlichen Trigeminus muskulatur abgezweigt hat"; its inner end is continuous by a tendon with the posterior digastric, the lower part of which is almost surrounded by the stylo-hyoid. A little higher up these two muscles diverge, the stylo-hyoid being affixed to the hyoid cartilage, the posterior digastric to the base of the skull. Both posterior digastric and stylo-hyoid receive branches from the N. facialis. (The earliest stages of development of these muscles were not described.) In stage 27 (embryo 92 mm. in greatest length) a jugulo-hyoideus is formed probably from a small part of the

posterior digastric. The stylo-hyoid has lost relation with the posterior digastric; it passes downwards, ending partially in a fascia lying at the posterior margin of the mylo-hyoid, partially by insertion into the hyoid.

Eschweiler (1911), who did not refer to the papers of Futamura and Kallius, gave an account of the development of the stapedius in the pig. He stated that the Anlage of the muscle is first visible in embryos of 13 mm. "Scheitel-Steisslänge," and has no "Abstammung von einer andern grossen Muskelgruppe." On the first development of Reichert's cartilage the muscle enters into relationship with it; it subsequently loses this and gains an attachment to the stapes. "Der Musculus Stapedius ist ein echter Abkömmling des Hyoidbogens und tritt erst sekundär mit dem Stapes, der dem periotischen Blastem enstammt, in Verbindung." The first basal attachment of the muscle is to the wall of the fossa stapedii s. Antrum petrosum laterale, and a certain rotation of the muscle takes place "in der Weise dass die Achse seiner Pyramide mit ihrem basale Ende nach hinten (aboralwarts) verschoben wird."

A re-investigation of the process of development in the pig showed the following: In a 13 mm. embryo there was no trace of an anterior digastric muscle. In a 14 mm. embryo it is being proliferated from the ventral surface of the hinder part of the intermandibularis, but is not separated from it, whilst the muscle Anlagen in the hyoid segment show the same structure as in the 15 mm. stage. In a 15 mm. embryo (figs. 55, 56) the anterior digastric is a little separated from the intermandibularis; it extends backwards and upwards on the lateral surface of the hyoid ventral constrictor portion of the muscle Anlagen in the hyoid segment, to about its middle. In the hyoid segment the hyoid bar consists of a continuous stapes, inter-hyale, and stylo-hyale (figs. 55–57) in a precartilaginous condition, and the latero-hyale extends upwards from the junction of the inter-hyale and stylo-hyale towards the auditory capsule, which shows no trace of a fossa stapedii or crista parotica. The muscle-Anlagen (figs. 56–58), not yet

separated into stapedius and hyoid ventral constrictor, extend as a continuous strip from behind the latero-hyale downwards and forwards lateral to the stylo-hyale, and then anterior to the stylo-hyale to the middle line just behind the posterior edge of the intermandibularis.

In a 17 mm. embryo the anterior digastric is quite separated from the intermandibularis. The stapedius portion of the hyoid muscle-Anlage is partially separated from the hyoid ventral constrictor, and some of its fibres are inserted into the back of the upper end of the stylo-hyale.

In a 19 mm. embryo the crista parotica is formed and the upper end of the latero-hyale is continuous with it. The stapedius muscle is now quite separated from the hyoid ventral constrictor ; it arises from the floor of the fossa stapedii and is inserted into the inter-hyale. The hyoid ventral constrictor has no dorsal attachment, its upper end lies just below the stapedius, and it extends downwards to a ventral aponeurosis. The anterior digastric extends laterally to Meckel's cartilage, whilst posteriorly it extends backwards and upwards on the lateral surface of the hyoid ventral constrictor, as in the 15 mm. stage.

In a 21 mm. embryo (figs. 61–63) the inter-hyale has disappeared and the stapedius is inserted into the stapes. The hyoid ventral constrictor has extended dorsally and its upper end is attached to the paroccipital process, and it has divided into dorsal and ventral portions. The dorsal portion is partially separated into jugulo-hyoideus and posterior digastric. The jugulo-hyoideus is inserted into the stylo-hyal cartilage. The lower end of the posterior digastric is continuous with the posterior end of the anterior digastric. The anterior end of the anterior digastric is attached laterally to the Anlage of the mandible. The dorsal end of the ventral portion of the hyoid ventral constrictor is attached to the stylo-hyale ; from this point it passes downwards to the transverse ventral aponeurosis.

In a 24 mm. embryo the only change is a slightly greater separation of the jugulo-hyoideus and posterior digastric. In

a 32 mm. embryo (fig. 64) the ventral end of the lower part
of the original hyoid constrictor has gained an insertion to
the external surface of the first branchial cornu, though
keeping its transverse aponeurosis to the muscle of the
opposite side—a stylo-hyoid muscle is thus formed.

The developmental phenomena in the rabbit are, for the
most part, similar to those in the pig. The anterior digastric
is proliferated from the intermandibularis, and grows backwards
medial (not lateral, as in the pig) to the hyoid ventral con-
strictor. The latter divides into dorsal and ventral portions,
the dorsal of which separates into posterior digastric and
jugulo-branchialis, whilst the ventral forms the stylo-hyoid.
The jugulo-branchialis becomes inserted into the first branchial
cornu; and the stylo-hyoid into the basibranchial, losing its
ventral transverse aponeurosis. The posterior digastric
becomes tendinous.

On the Primary Form of the Posterior Digastric
in Mammals.—Bijvoet's conclusion, from an examination of
the adult condition of the stylo-hyoid and posterior digastric
in many classes of Mammals, was that, primitively, " sich vom
Schädel zum Zungenbein, oder besser zu einer Bindegewebs-
lager, das sich ventral am Hyoid heftet, eine Muskelmasse
erstreckt, die vom N. facialis innerviert wird." " Den hier
beschriebenen Zustand begegnen wir bei Echidna und
Ornithorhynchus. Der M. styloideus repräsentiert die einheit-
liche Muskelmasse, welche vom Schädel entspringt und die
ventrohyoideale Bindegewebsmasse sich festheftet."

The developmental phenomena in Ornithorhynchus,
Echidna, Dasyurus, pig, and rabbit show that a still more
primitive condition of the muscle is one in which it is attached
dorsally to the hyoid bar and passes ventrally to a transverse
aponeurosis, forming a hyoid ventral constrictor or inter-
hyoideus. This condition in Dasyurus, pig and rabbit is
succeeded by one in which the muscle loses its dorsal attach-
ment to the hyoid bar, and, extending dorsally, gains a new
one to the skull—to either the paroccipital or mastoid process.
The more primitive condition, however, persists in Echidna,

in which animal Toldt described the M. styloideus[1] as arising from the stylo-hyale close to its cranial end.[2] This I can confirm (vide fig. 38); it arises from the upper end of the stylo-hyale and from the latero-hyale, lapping round the insertion of the levator hyoidei.[3]

Comparison of this adult condition with stages 47 and 50 (fig. 37) shows that the origin of the muscle has spread slightly up the hyoid bar. The muscle also arises from the stylo-hyale in Manis pentadactyla (fig. 43).

It is doubtful whether this primitive condition exists in any Mammals other than these two. It is true that Kohlbrugge described the posterior belly of the digastricus as arising from the styloid process in the Marsupials, Cuscus orientalis and maculatus, Paradoxurus hermaphrodita, and Macropus brunii. But in Cuscus maculatus, Bijvoet described and figured the posterior digastric as arising from the paroccipital process—an occurrence which suggests that in the specimen examined by Kohlbrugge the condition was one of division of a posterior digastric s. hyoid ventral constrictor into dorsal and ventral portions (vide pp. 617, 618), of which only the ventral was described. The same explanation possibly applies to the other Marsupials described by Kohlbrugge.

A condition intermediate between that of Echidna and the usual one of attachment to the skull is present in Ornithorhynchus, where Toldt described the muscle as arising from the stylo-hyale and hinder wall of the cartilaginous external auditory meatus.[4]

[1] I employ the nomenclature of Schulman. Toldt names the muscle the " stylohyoideus,"—a terminology suggesting a homology with the " stylohyoideus " of the pig and rabbit, which is developed from the ventral part only of the hyoid ventral constrictor (vide pp. 617, 618).

[2] Bijvoet described the muscle as arising " unmittelbar hinter dem äusseren Gehörgange."

[3] No representative of a possible upper portion of the muscle was present.

[4] Bijvoet described it as arising " von der Schädelbasis und empfängt accessorische Ursprünge vom äusseren Gehörganges." Schulman did not state its origin.

The hyoid ventral constrictor or styloideus muscle, unconnected with the anterior digastric to form a digastricus, passes downwards to a ventral aponeurosis connecting it with its fellow below the basibranchial and ventral ends of the hyoid bars. This condition is present in stages A–D of Dasyurus, in 14 mm. embryos of the pig, and is preserved to the adult condition in Echidna and Ornithorhynchus (Schulman), and in Manis. It is also present in some of those Edentates, i. e. Dasypus villosus, Tolypentes tricinctus, and Tatusia novemcincta (Toldt), in which a sterno-mandibularis exists. The latter muscle (vide p. 625) is formed by the union of the anterior digastric with the sterno-hyoid, and the transverse aponeurosis of the hyoid ventral constrictor s. posterior digastric lies dorsal to the longitudinal muscle.

In some Edentates, e. g. Bradypus tridactylus, the anterior end of the sterno-hyoid is in part attached to the posterior edge of the transverse aponeurosis of the hyoid constrictor s. posterior digastric (Bijvoet).

In some Edentates the hyoid ventral constrictor s. styloideus s. posterior digastric divides into dorsal and ventral portions, the former taking origin from the skull and inserted into the stylo-hyal, the latter arising therefrom and passing to a ventral aponeurosis.

Thus in Bradypus tridactylus (Bijvoet) the posterior digastric arises from the mastoid process and passes to a ventral aponeurosis (fig. 40)[1]; whilst in Bradypus marmoratus (fig. 41) the muscle is divided into dorsal and ventral portions. The stapedius muscle is present. A similar condition was found in a 30 mm. embryo, though the separation into dorsal and ventral portions was not quite complete.

Toldt described the posterior digastric in Dasypus villosus as arising from the mastoid; its tendon broadens and unites with the posterior border of the mylo-hyoid. In Dasypus novemcincta (embryo 30 mm.) the posterior digastric is partly divided into dorsal and ventral portions,

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[1] This figure of Bijvoet is reproduced for comparison with one of Bradypus marmoratus.

inserted into and arising from the stylo-hyale (fig. 42). A sta-
pedius of usual origin and insertion is present.

Mackintosh described the posterior digastric of Choloepus as
arising from the stylo-hyale, but did not mention the existence
of any muscle arising from the mastoid and inserted into the
bar. Such a muscle, however, is pictured in a figure of
Schulman under the title of mastoideo-hyoideus.

Toldt stated that the posterior digastric was absent in
Tamandua, but described and pictured a " stylo-hyoideus,"
innervated by the N. facialis, taking origin from the os tym-
panicum and inserted into the cranial end of the hyoid cornu,
and also described a portion of the intermandibularis as
arising from the hyoid cornu. These two muscles are in all
probability the dorsal and ventral portions of the posterior
digastric. This identification is rendered all the more certain
by his statement that there is an anastomosis between the
mylohyoid branch of the fifth and the seventh nerves.

Owen described three muscles in Myrmecophaga jubata
—a " stylohyoideus " passing from the petro-hyoid to the
stylo-hyale, a " cerato-hyoideus " passing from the cerato-
hyale to a commissural tendon with a slip to the sterno-
mandibularis muscle, and a " constrictor salivaris " passing
from the cerato-hyale downwards over the salivary reservoir to
the commissural tendon and blending with the back of the
intermandibularis. It is probable from his description and
figures that his " stylo-hyoideus " and " cerato-hyoideus " are
the dorsal and ventral portions of a divided posterior digas-
tric. Leche stated that the constrictor salivaris is innervated
by the N. mylo-hyoideus and is to be regarded as a differ-
entiated part of the intermandibularis.

The condition of the hyoid ventral constrictor is thus
variable in Edentates, even in closely related species. Thus it
is undivided in Bradypus tridactylus, divided into upper
and lower parts in Bradypus marmoratus; undivided in
Dasypus villosus, divided in Dasypus novemcincta.
And in at least one Marsupial—Cuscus maculatus—the
condition is variable within the same species (vide p. 619).

A sterno-mandibularis may occur with either condition, e. g. with an undivided hyoid ventral constrictor in Tolypeutes tricinctus and Dasypus villosus, with a divided one in Dasypus novemcincta, Myrmecophaga and Tamandua.

In the pig, as described above, the hyoid ventral constrictor divides into dorsal and ventral portions. The dorsal portion separates into two muscles—the jugulo-hyoideus and the posterior digastric. The jugulo-hyoideus is inserted into the stylohyal. The ventral end of the posterior digastric unites with the posterior end of the anterior digastric and forms the digastric muscle. The ventral portion of the hyoid ventral constrictor becomes the stylo-hyoid; it arises from the stylo-hyal and passes to a ventral aponeurosis uniting it with its fellow, and also gains an insertion to the side of the first branchial cartilage.

In the rabbit the sequence of events is, but for two differences, the same. The jugulo-hyoideus gains an insertion to the first branchial cartilage, becoming the jugulo-branchialis. The ventral portion of the constrictor becomes the stylo-hyoid and is inserted into the basibranchial; the ventral aponeurosis disappears.

In both animals the posterior digastric becomes tendinous and has no nerve-supply, but in the embryo a branch of the N. facialis nerve passes to it.

On the Formation of the Anterior Digastric Muscle.—Consideration of the adult form and innervation of the anterior digastric muscle led Gegenbaur, Ruge, Fürbringer, Schulman and Bijvoet to the opinion that it is derived from the intermandibularis s. mylo-hyoid, and the observations of Kallius, though he did not observe the earliest stages of the process, tended to confirm this view. Chaine stated that the digastric muscle is due to longitudinal division of a muscle extending from the sternum to the mandible into inner and outer portions, the inner forming the genio-hyoid and sterno-hyoid, the outer the digastricus.

Futamura stated that in man and pig the anterior digastric is due to a forward extension to the jaw of the muscle blastema

which gives rise to the stapedius, stylo-hyoid, and posterior digastric. This forward growth receives a secondary innervation from the fifth nerve. These statements were adversely criticised by Bijvoet.

The above-recorded observations show that the depressor mandibulæ anterior of Ornithorhynchus, and its homologue, the anterior digastric of Dasyurus, pig and rabbit, are formed by proliferation from the ventral surface of the intermandibularis.

On the Formation of the Digastric Muscle.—Schulman, followed by Bijvoet, came to the conclusion that the primitive condition of the anterior digastric muscle is indicated by the depressor mandibulæ anterior of Echidna and Ornithorhynchus,[1] viz. a sheet of approximately transverse fibres below the intermandibularis, attached laterally to the lower jaw; that this transverse sheet became more longitudinal in direction, and that its hind end—the original inner end— gained an attachment to the transverse aponeurosis of the posterior digastric muscle. A condition similar to that of Bradypus tridactylus would result, and from this all the varied forms of a digastricus verus and digastricus spurius can be easily derived.

Toldt's theory of the phylogenetic development of the digastricus muscle differs from that of Schulman and Bijvoet in some particulars, and is as follows. The anterior digastric " besitzt ursprünglich, gleich dem M. mylohyoideus, den Character und die Bedeutung eines Eingeweidemuskels; er ist die äussere Langsfaserschichte des Mundhöhlenbodens, angelegt der Querfaserschichte desselben, dem M. mylohyoideus." This longitudinal muscle unites with a hinder longitudinal muscle innervated by the twelfth, to form a continuous longitudinal muscle—the sterno-mandibularis, extending from the sternum to the lower jaw. This condition is

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[1] Schulman, " führt die Vereinigung des M. depressor mandibulæ anterior mit vom N. facialis versorgten M. depressor mandibulæ posterior oder richtiger einem hyoidealen Componenten des M. constrictor zur Bildung eines wirklichen M. digastricus."

present in Myrmecophaga, Tatusia, Dasypus villosus. In Tolypeutes tricinctus a tendinous inscription is present between the anterior and posterior portions, and is attached to the hyoid. A sterno-hyoideus lies dorsal to the posterior portion. The posterior digastric ends in a transverse aponeurosis in intimate connection with the posterior margin of the inter-mandibularis. In Bradypus tridactylus there is a tendinous inscription; the anterior portion forms an anterior digastric and the posterior portion a true sterno-hyoideus in that whilst its median portion is attached to the inscription its lateral portion is attached to the first branchial bar. The posterior digastric is attached to the lateral end of the inscription. A digastric muscle is thus formed. This condition is one intermediate between those described above and that found in higher Mammals.

The following observations bear on this question. In embryos of Amphibia and Sauropsida, and also in the rabbit and pig, the hypobranchial spinal muscles—formed by downgrowths from two or more anterior body myotomes—form a longitudinal cell column, the anterior end of which, growing forward dorsal to the inter-hyoideus s. hyoid ventral constrictor and intermandibularis to the front end of Meckel's cartilage, divides into two at the level of the second or first branchial cartilage, the part in front forming the genio-hyoid, the part behind the sterno-hyoid. In the rabbit and pig this primitive sterno-hyoid develops into sterno-hyoid, sterno-thyroid, thyro-hyoid, omo-hyoid.[1]

It would follow that any connection of the anterior end of the sterno-hyoid with a muscle lying ventral to the inter-mandibularis s. mylohyoid is a secondary phenomenon.

Further, in a 30 mm. embryo of Dasypus novemcinctus the following condition was found: The sterno-mandibularis had a tendinous intersection at the level of the hind edge of the intermandibularis. The sterno-thyroid was attached to the basibranchial and thyroid cartilage by two separate slips. A thyro-hyoid extended from the thyroid to the first branchial

[1] Details of this development will be given in a later paper.

bar.   The genio-hyoid and hyo-glossus arose from the basi-branchial and first branchial bar and extended forward to the anterior end of the lower jaw and tongue respectively.   A branchio-hyoid s. cerato-hyoid muscle, between the first branchial and hyoid bars, was also present.   The posterior digastric consisted of upper and lower parts, the upper extending from the mastoid to the hyoid bar, the lower from the hyoid bar to an aponeurosis immediately behind and continuous with the intermandibularis.   A stylo-glossus arose from the hyoid bar.

In this specimen, then, there was evidence of fusion between a sterno-hyoid and anterior digastric to form a sterno-mandibularis.   In the other Edentates possessing a sterno-mandibularis Toldt does not state whether the muscle was investigated microscopically.

These phenomena tend to show that the connection of the sterno-hyoid to the transverse aponeurosis of the posterior digastric is a secondary phenomenon and related to its non-attachment to the first branchial bar, and suggest that its fusion with the anterior digastric to form a sterno-mandibularis is also related to the same occurrence.

The theory of Toldt, again, quite fails to account for the fact that in Dasyurus the anterior digastric, when first formed, grows out transversely just as in Ornithorhynchus, and only subsequently takes up a longitudinal direction.

The theory of Toldt is thus open to many objections, and may probably be rejected in favour of that of Schulman and Bijvoet.

The above observations, however, show that there are two main varieties of a digastric muscle in mammals.

(1) The anterior digastric becomes connected with the ventral end of the hyoid ventral constrictor s. stylo-hyoideus; no stylo-hyoid muscle is present.   This occurs in Marsupials and those Edentates in which a digastric is formed.   There are two sub-varieties : (a) the hyoid ventral constrictor remains undivided in most Marsupials and some Edentates, e. g. Bradypus tridactylus; (b) the hyoid ventral con-

strictor divides into dorsal and ventral portions, and the anterior digastric becomes connected with the lower end of the ventral portion, e. g. Bradypus marmoratus, Cholœpus, and probably the Marsupials examined by Kohlbrugge (vide p. 619).

(2) The hyoid ventral constrictor divides into dorsal and ventral portions, and the anterior digastric becomes connected with the lower end of the dorsal portion; the ventral portion forms, as a rule, the stylo-hyoid muscle. This condition is present in all Eutheria. Phylogenetically, it is probably derived from sub-variety (b) above, rather than from sub-variety (a). Such a digastric muscle may be of many different forms—classified by Bijvoet into " digastricus verus " and " digastricus spurius."

The posterior digastric of this latter class of Mammals is partially or wholly—according to whether a mastoideo- s. jugulo-hyoideus s. jugulo-branchialis is or is not developed—homologous with the dorsal portion of the posterior digastric of these Edentates, in which division into dorsal and ventral portions takes place. The mastoideo- s. jugulo-hyoideus preserves the ancient insertion of the muscle. The stylo-hyoideus is homologous with the ventral portion. It may pass to the middle line and join its fellow, thus preserving the primitive condition, as in Cynocephalus (Bijvoet); or, keeping the transverse aponeurosis it may gain an additional insertion to the branchial cornu, as in the pig; more commonly, however, the aponeurosis disappears and the muscle is inserted into the basibranchial or its branchial cornu, as in the rabbit. The origin of the muscle often extends upwards on the stylo-hyal and may even reach the skull, so that it is overlapped by the posterior digastric, and the primitive positions of the two muscles are obscured.

According to Bijvoet a stylo-hyoid muscle is absent in Erinacæus among Insectivora, the Cheiroptera, and some Mustelidæ, but he also states that in some Erinacæus some bundles of the posterior digastric stream to a thin membrane which passes inwards to the middle line ventral to the hinder

part of the mylo-hyoid, whilst in Cheiroptera and Mustelidæ some of the hinder bundles of the mylo-hyoid arise from the hyoid. A representative of the ventral part of the hyoid constrictor is thus present, though not forming a stylo-hyoid muscle.

In view of these phenomena of comparative anatomy and embryology it is probable that the accounts given by Rouvière and Futamura of the development of the stylo-hyoid of man— that it is an outgrowth of the posterior digastric—are in need of revision.

On the Reported Instances of a Monogastric Digastric Muscle Inserted into the Lower Jaw.— Kohlbrugge found this condition present in one specimen of Hystrix with a fifth nerve innervation, and found a two-bellied condition in another specimen. The former was explained by Schulman as one in which an anterior digastric had extended backwards.

In Manis javanica Kohlbrugge described the digastric as a simple muscle extending from the " hinteren Schädelseite zum Kieferwinkel," whilst in Manis macrura Windle and Parsons found the digastric "inserted into the lower jaw as far as halfway to the symphysis." In Manis pentadactyla (fig. 43) there is a muscle, with the insertion described by Windle and Parsons, arising partly from the mastoid and partly by fibres which are continuous with the platysma in the neck, i. e. an auriculo-mandibularis. The posterior digastric is present—in the form of a hyoid ventral constrictor, arising from the stylo-hyal and innervated by the N. facialis; its anterior edge, ventrally, is continuous with the posterior edge of the intermandibularis. The anterior digastric muscle is absent.

In Tatusia and Dasypus a monogastric muscle, attached below to the mandible, was described by Macalister, but later investigations by Toldt show that a posterior digastric passing to a ventral aponeurosis is present, so that the first described muscle is an auriculo-mandibularis.

In Orycteropus, Humphry described the digastric as

extending " from mastoid process to angle and lower margin of jaw." Subsequently, Chaine stated that "au lieu du muscle que signale Humphry j'ai trouvé une formation mi-tendineuse mi-musculaire qui parassait en tenir lieu," probably, therefore, an auriculo-mandibularis; and he also depicted, though not describing, an additional muscle named digastric.

It is not known whether a true posterior digastric exists in Cyclothurus and Chlamydophorus. In the latter a small muscle passing from the bulla tympani to the mandible was found by Macalister, but not by Hyrtl.

The case of Tamandua is considered above (p. 621).

The existence of a monogastric muscle homologous with the posterior digastric and inserted into the lower jaw is thus very doubtful. The instances which have been described are probably cases of an auriculo-mandibularis—which is derived from the platysma (vide pp. 632, 634).

On the Primary Form of the Stapedius Muscle in Mammals.—The stapedius muscle in early post-embryonic stages of Dasyurus arises from the outer surface of the auditory capsule and is inserted into the upper part of the stylohyale, forming a levator hyoidei. Its origin subsequently shifts to the lower edge of the developing crista parotica, and then to the floor of the fossa stapedii, whilst its insertion shifts to the stapes. The initial stage present in Dasyurus is, in part, passed over in the rabbit and pig, for in them the first dorsal attachment of the muscle is to the floor of the fossa stapedii; on the other hand, its first ventral attachment is to the upper end of the stylo-hyale, subsequently shifting to the inter-hyale and then to the stapes as in Dasyurus. The shifting of insertion from the inter-hyale to the stapes takes place with the disappearance of the inter-hyale in all three animals. The primary form of the stapedius as a levator hyoidei is present in 8·5 mm. embryos of Ornithorhynchus, and in stage 47 of Echidna, and in them persists into adult life. It is the muscle which was termed " mastoideo-hyoideus " by Schulman and Bijvoet.

This identification affords an explanation of the statements of Huxley, Eschweiler and Fürbringer, that a stapedius muscle, i. e. one of the usual mammalian type, is absent in Monotremes, and also of the method of innervation. In non-Monotreme Mammals the nerve to the stapedius is given off from the N. facialis proximal to the chorda tympani. Now in Echidna—according to Schulman—the mastoideo-hyoideus is innervated by several fine twigs from the N. facialis, some of which are given off proximal to, and some with, the chorda tympani.

The relations of the N. facialis to the levator hyoidei s. stapedius in Echidna are different from those in Ornitho-rhynchus, Dasyurus, pig and rabbit. In the latter four animals the nerve passes backwards lateral to the muscle and then downwards. Earlier stages of Ornithorhynchus and Dasyurus were not available, but in the rabbit and pig this condition is preceded by one in which the nerve passes out-wards dorsal to the Anlage of the stapedius and hyoid ventral constrictor and then downwards. The levator hyoidei s. stapedius thus extends upwards on the inner, medial, side of the nerve to gain a dorsal attachment to the outer surface of auditory capsule or to the floor of the fossa stapedii.

In stage 47 of Echidna the nerve passes backwards in the sulcus facialis dorsal to, and separated by the incurving crista parotica from the levator hyoidei which arises from it. In stage 50 (figs. 36 and 37) the origin of the levator hyoidei has extended outwards to the base of the crista parotica, and in the adult (fig. 38) to the outer surface of the auditory capsule. The N. facialis consequently passes backwards medial to the muscle, whereas in other Mammals it passes backwards lateral to it.

It would thus appear that the name " mastoideo-hyoideus " has been applied by various investigators to three muscles of differing origin and morphological nature : (1) to a stapedius muscle which has preserved a levator hyoidei stage, as in Monotremes ; (2) to the dorsal portion of a hyoid ventral constrictor which has divided into dorsal and ventral portions,

as in Bradypus marmoratus, Dasypus novemcincta,
Cholœpus didactylus; and (3) to the part of the dorsal
portion of a hyoid ventral constriction which is inserted into
the stylo-hyal, as in the pig.

The name " stylo-hyoideus " was applied by Owen and by
Toldt to (2) above, and also by Toldt to the hyoid ventral
constrictor of Monotremes.

On the Homologies of the Posterior Digastric
Muscle in Non-Mammals.—According to Gegenbaur and
Ruge the posterior digastric is homologous with the depressor
mandibulæ of lower vertebrates, and the latter thought that
the change of insertion, from mandible to hyoid, was related
to the formation of the new squamoso-mandibular joint.
Fürbringer and Bijvoet rejected this theory, the former
being of opinion that the old depressor mandibulæ was either
altogether gone or would be found as a rudimentary structure
attached to the malleus, the latter finding it difficult to under-
stand how the insertion of a depressor mandibulæ should be
transferred to the hyoid.

Bijvoet's theory was that the posterior digastric is derived
from a ventral constrictor of the hyoid segment, and is serially
homologous with the intermandibularis s. mylohyoid of the
mandibular segment. This becomes all the more probable
from the investigations detailed in this paper, which show
that the primary upper attachment of the muscle is to the
hyoid bar.

The serial homology is well brought out in fig. 4, which,
being drawn from a slightly oblique section of Dasyurus in
stage B, shows the posterior digastric on one side and the
intermandibularis on the other.

On the Homologies of the Stapedius in Non-
Mammals.—Fürbringer was of the opinion that the de-
pressor mandibulæ of non-mammals has entirely disappeared
in mammals, or will be found as a rudiment attached to the
malleus—the hind end of the primitive jaw. No such rudi-
ment, however, was found in the embryos investigated.

Gaupp stated that the disappearance of the depressor is a

fact: " Der Schwund des alten M. depressor mandibulæ bei den Säugern und damit der Wechsel in dem Muskelmechanismus bei den Oeffnung des Mundes sind Tatsachen."

But, perhaps, after all, the muscle has not disappeared. It has been shown above that the stapedius muscle of Mammals is primarily a levator hyoidei arising from the outer wall of the auditory capsule and inserted into the upper part of the hyoid bar. Now, the depressor mandibulæ of Dipnoi, Urodela[1] and Sauropsida, as I have shown in a former paper, is at first a levator hyoidei inserted into the hyoid bar—a condition which persists in Protopterus—and only subsequently becomes attached to the hind end of Meckel's cartilage. It may be inferred that the stapedius muscle of Mammals is homologous with the levator hyoidei of Urodela and Sauropsida though not with its later stage of depressor mandibulæ—there being no developmental or other evidence that it ever gained that secondary attachment.

The origin of the superficial facial and platysma muscles has been investigated by the methods of comparative anatomy and by direct observation of the phenomena of development.

Rabl stated that the platysma develops in the territory of the hyoid arch, grows forward, and upwards behind the Anlage of the external ear, and gives rise to the whole of the mimetic facial musculature and that of the epicranium. He did not further particularise exactly where and how it develops, nor the relationship of the Anlage of the platysma to the stapedius, posterior digastric and stylo-hyoid.

Futamura stated that in man and pig the facialis musculature is derived from an aggregate of myogenic cells round the motor facial nerve. The proximal part consists of two layers separated by the N. facialis, of which the lateral forms the Platysma colli.

There is no trace of the superficial facial and platysma

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[1] The condition in the Anura is more complicated, the levator hyoidei of the tadpole forming only the superficial portion of the depressor mandibulæ of the adult form.

muscles in Stages A and B of Dasyurus. Indifferent meso-
blast cells lie between the lateral surface of the hyoid ventral
constrictor and the epidermis (fig. 5). In stage C (fig. 13)
the Anlage of the sphincter profundus and platysma muscles
is developed in this mesoblast, about midway between the
lateral surface of the hyoid ventral constrictor and the
epidermis, and extending dorsally lateral to the submaxillary
gland. No evidence is afforded by the sections that these
embryonic muscles are proliferated or delaminated from the
hyoid ventral constrictor—they are developed in the sub-
epithelial mesoblast. In stage D (fig. 18) the embryonic
muscle-cells are better developed and have spread forwards
into the mandibular segment. In stage E (figs. 20 and 21)
they have spread still further forwards in the mandibular
segment, and down the neck. In the mandibular and hyoid
segments they show evidence of division into two layers—
an inner, of cells elongated in the transverse plane, the
sphincter profundus ; and an outer, of cells elongated in the
longitudinal plane, the platysma. In stage F (figs. 22 and
23) these two sheets are more widely separated, and the
platysma has spread dorsally over the temporal muscle and
forwards, forming the Anlage of the sphincter palpebrarum ;
whilst the sphincter profundus does not extend dorsally
above the level of the zygomatic arch. The sphincter
profundus is not developed behind the external ear ; its hind-
most fibres form the depressor auris s. pars auricularis of
the constrictor profundus. The Anlage of the auriculo-
mandibularis is formed as a forward and downward growth
from the platysma sheet, opposite the incus (fig. 23). In
stage H (fig. 28) and in the 10 mm. stage of Didelphys
aurita (fig. 39) it has separated from the platysma and
extended forwards and backwards; its anterior end is
attached to the posterior edge of the mandible and its
posterior end to the internal surface of the aural cartilage.

In stage H the buccinator and maxillo-labialis are formed
from the sphincter profundus.

The muscles of the external ear begin to be differentiated

in stage F, and are well marked in stage H; the depressor
auris, as stated above, is developed from the sphincter pro-
fundus; all the other muscles are developed from the platysma
sheet, which is solely present behind the ear, and in front
solely present above the zygomatic arch. They consist of
sculutaris, post-auricularis, auriculo-occipitalis, three little
muscles on the outside, and two—the transversus and obliquus
—on the medial side of the cartilage.

These developmental phenomena in Dasyurus confirm the
opinion of Boas and Paulli that the orbicularis palpebrarum
is derived from the platysma sheet, the opinion of Ruge that
the buccinator is derived from the sphincter profundus, and
show that the maxillo-labialis is derived from the sphincter
profundus.

In an 8·5 mm. embryo of Ornithorhynchus the Anlage of
the sphincter superficialis and platysma muscles is present
in the hyoid segment outside the hyoid ventral constrictor
(figs. 29–33). It has spread forwards into the mandibular
segment, and backwards into the neck. It shows slight
indications of separating into the sphincter superficialis and
platysma sheets.

In 14 mm. embryos of the pig there is no trace of the
Anlage of the sphincter profundus and platysma muscles;
the space between the outer surface of the Anlage of the
stapedius and hyoid ventral constrictor and the epiblast is
packed with mesoblast, in which no trace of differentiation
can be seen. In 15 mm. embryos (figs. 56, 58) the Anlage
of the sphincter profundus and platysma can be seen in the
ventral part of the hyoid segment, close to the epiblast, and
separated by an interval filled with indifferent mesoblast cells
from the Anlage of the hyoid ventral constrictor.

The auriculo-mandibularis muscle — either muscular or
tendinous—is present in many Mammals. It takes origin
from the cartilaginous portion of the external auditory meatus
or cartilage of the outer ear, and is inserted into the ramus
ascendens of the mandible (Bijvoet). It is innervated by the
N. facialis, either by a branch from the rami auriculares

posteriores, e. g. Canis (Chaine), or by a more distal branch than that for the posterior digastric, e. g. Tatusia, Marsupials (Bijvoet).

Two views have been advanced as to the derivation of the muscle. Chaine held that it is homologous with the depressor mandibulæ of lower Vertebrates; Fürbringer and Bijvoet that it is a derivation of the facial musculature. The above-described phenomena in Dasyurus prove the correctness of the latter theory; they show that it is derived from the platysma. The muscle is one which was identified by some observers as a single-bellied digastric inserted into the lower jaw, e. g. in Dasyurus, Tatusia, Manis. This question is discussed above (pp. 627, 628).

On the Derivation of the Muscles of the External Ear.—The comparative anatomy of the muscles of the external ear in Mammals has been investigated by Ruge, Baum and Kirsten, and by Boas and Paulli; their development by Killian, Dobers and Futamura.

Ruge was of the opinion that all the auricular muscles were derived from the platysma; none from the sphincter profundus.

Boas and Paulli agreed with this opinion, except in regard to the depressor auris, which they described as the pars auricularis of the sphincter profundus.

Killian stated that the muscles in front of the ear—attrahens, attolens (anterior part), helicis major, helicis minor, tragicus—were derived from an upgrowth of the platysma in front of the ear; and those behind—retrahens, hinder part of the attolens, obliquus, transversus—from the hinder superficial layer of the dorsal portion of the hyoid arch musculature.

Dobers stated that in the pig the muscles develop from two groups which "zwar gemeinschaftlicher Abstammung sind, sich jedoch sehr frühzeitig trennen." One group lies from the very beginning caudo-dorsal to the ear; the second group lies more ventral, and can be called the embryonic platysma, for it also gives rise to the platysma myoides.

From the second group develop the tragicus, adductor auris inferior and externus and helicis major; from the first group the other muscles.

Futamura's statements differ much from the above; they are that, in the pig, the scutularis, helicis major and minor are developed from the platysma, and the other muscles from the sphincter auris, which is derived from the sphincter colli.

In Dasyurus the superficial muscle sheet spreads upwards from the site of its formation in the mesoblast external to the hyoid ventral constrictor, and divides into two layers, each consisting of embryonic muscle-cells easily distinguishable from one another and from surrounding mesoblast. The sphincter profundus does not spread above the zygomatic arch, nor is it present behind the ear. Only the depressor auris is developed from the sphincter profundus; all the other muscles are derived from the platysma sheet. This method is also evident in Didelphys aurita. The embryological phenomena in these two Marsupials thus confirm the theory of Boas and Paulli.

The development of the cervical portion of the platysma by gradual descent from the hyoid region is of special interest owing to the discovery by Kohlbrugge that in many Marsupials and Edentates [1] the R. cervicis of the N. facialis is entirely replaced by the Nn. cutanei of the cervical nerves so that it is entirely restricted to the face. Kohlbrugge advanced the following theories in explanation: "Entweder täuscht der R. colli des Menschen nur eine primitive Lage vor und erreichte die Halsregion nur durch ein Herabrücken der Muskulatur welches bei den Marsupialieren dann noch nicht bei den Monotremen wohl eingetreten ware, oder wir müssen eine secundäre Reduction dieser Theile bei Marsupialiern (und Manis) annehmen, die Gegend welche sonst der Facialismuskulatur angehörte wäre dann von den spinalen Nerven und ihrer Muskeln erobert worden." He was inclined to accept the latter theory.

[1] Cuscus orientalis, Cuscus maculatus, Paradoxurus hermaphrodita, Macropus brunii, Manis javanica.

There is a third possibility—that the superficial muscles of the neck may be of facial origin and receive a secondary innervation by cervical nerves ; this is confirmed by the developmental phenomena in Dasyurus, where the platysma originates in the hyoid region and spreads down the neck.

This phenomenon is one which can be assimilated with others occurring in the muscles of the head. I have previously given a list of thirteen in various Vertebrates, which appear to be referable to the law that if a muscle grows into one or more neighbouring segments, that portion tends to be innervated by the corresponding nerve or nerves.

It is noteworthy that in Monotremes (McKay, cited by Kohlbrugge) the cutaneous muscles of the neck are innervated by both the N. facialis and the first four cervical nerves—i. e. a condition exists re innervation intermediate between that present in Marsupials and that in which the innervation is solely by the seventh nerve. It is similar to the double innervation of the trapezius by the N. accessorius and cervical nerves.

Kohlbrugge also found that in Marsupials the cervical nerves innervate the Platysma sheet in front of the ear, the N. facialis only innervating the sphincter profundus. The cervical nerves thus spread beyond their segmental territory and innervate muscles which did not originate in it. This must be a very rare occurrence as regards motor nerves, though it is parallelled by the N. vagus.

On the Homologies of the Sphincter superficialis s. colli, Platysma, and Sphincter profundus, in Non-Mammals.—Ruge, who investigated the adult form of the hyoid muscles in all classes of Vertebrates, stated that the sphincter colli of Monotremes is homologous with the muscle C_2vd of Selachians, and the Platysma group with the muscle C_2md.

Now in Ornithorynchus, Dasyurus and pig these muscles are formed from a common Anlage developed in the mesoblast outside and not as an extension backwards of the hyoid ventral constrictor—so that the homologies suggested by Ruge

appear impossible.   Similarly, they cannot be homologous
with the constrictor colli of Sauropsida, for this is developed
as an extension backwards of the levator hyoidei and inter-
hyoideus, nor with the backward extension of the inter-hyoideus
which occurs in many Amphibia.

It is therefore doubtful whether these skin muscles of
Mammals have any homologies in non-mammals; they have
probably been developed within the Mammalian phylum.

The phenomena recorded above suggest (1) that the
sphincter profundus, platysma, and sphincter superficialis
muscles have been developed within the Mammalian phylum.
(2) That the other muscles developed in the hyoid segment
are derived from (a) a levator hyoidei, arising from the outer
surface of the auditory capsule and inserted into the upper part
of the hyoid bar; (b) a ventral constrictor or inter-hyoideus,
arising from the hyoid bar and passing to a median raphé.

When the primitive conditions of the mandibular and hyoid
muscles in Mammalia are compared with those of Amphibia
and Sauropsida, it becomes clear that they point to the same
conclusion as that arrived at by Fürbringer in his " Abstam-
mung der Säugethiere "—that Mammals are descended from
a pro-amphibian stock.

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EXPLANATION OF PLATES 38–45,

Illustrating Dr. F. H. Edgeworth's paper " On the Development and Morphology of the Mandibular and Hyoid Muscles of Mammals."

ABBREVIATIONS.

Ala temp. Ala temporalis. *alisphen. b.* Alisphenoid bone. *An. hy. gloss. & styl. gloss.* Anlage of the hyo-glossus and stylo-glossus muscles. *An. pal. & pty. b⁵.* Anlage of palate and pterygoid bones. *An. of intermand. m.* Anlage of intermandibularis muscle. *An. of mast. ms* Anlage of masticatory muscles. *An. par. b.* Anlage of parietal bone. *An. Sph. sup. & platys.* Anlage of sphincter superficialis and platysma muscles. *An. stap. & hy. v. cons.* Anlage of stapedius and hyoid ventral constrictor. *An. tens. pal. & tens. ty.* Anlage of tensor veli palatini and tensor tympani muscles. *An. zygo. & mass.* Anlage of zygomatico-mandibularis and masseter muscles. *Annulus m.* Annulus muscle. *ant. dig. m.* Anterior digastric muscle. *aud. cap.* Auditory capsule. *aud. epith.* Epithelium of auditory vesicle. *aur. mand. m.* Auriculo-mandibularis muscle. *aur. temp. n.* Auriculo-temporal nerve. *basibr. c.* Basibranchial cartilage. *1 br. bar.* First branchial bar. *br. hy. m.* Branchio-hyoid muscle s. cerato-hyoideus s. inter-hyoideus. *br. IX to g. s. p.* Branch of ninth nerve to great superficial petrosal nerve. *bucc. m.* Buccinator muscle. *buccal n.* Buccal nerve. *chorda ty.* Chorda tympani nerve. *com. br. IX to VII.* Communicating branch of ninth nerve to seventh nerve. *cons. ph. m.* Constrictor pharyngis muscle. *crista par.* Crista parotica. *cut. br. mylohy. n.* Cutaneous branch of the mylohyoid nerve. *dep. auris m.* Depressor auris muscle. *dep. mand. ant. m.* Depressor mandibulæ anterior muscle. *detrah. mand. m.* Detrahers mandibulæ muscle. *E. A. M.* External auditory meatus. *ext. pty. m.* External pterygoid muscle. *ext. rect. m.* External rectus muscle of eye. *Eust. tb.* Eustachian tube. *Gass. g.* Gasserian ganglion. *gen. gloss. m.* Genio-

glossus muscle. *gen. gloss. post. ext.* Genioglossus posticus externus muscle. *gen. gloss. post. int.* Genioglossus posticus internus muscle. *gen. hy. m.* Genio-hyoid muscle. *genic. g.* Geniculate ganglion of seventh. *g. s. p.* Great superficial petrosal nerve. *ham.* Hamulus. *hy. ep. m.* Hyo-epiglotticus muscle. *hyogloss. m.* Hyo-glossus muscle. *hy. v. cons.* Hyoid ventral constrictor muscle. *hy. v. cons. d. p.* Hyoid ventral constrictor muscle, dorsal portion. *hy. v. cons. v. p.* Hyoid ventral constrictor muscle, ventral portion. *inf. d. ling. & mylohy. n.* Trunk which divides into inferior dental, lingual and mylo-hyoid nerves. *int. car. a.* Internal carotid artery. *int. hyal.* Inter-hyale. *interart. men.* Interarticular meniscus of jaw joint. *intermand. m.* Intermandibularis muscle. *int. pty. m.* Internal pterygoid muscle. *jug. hy. m.* Jugulo-hyoideus muscle. *lam. asc. a. t.* Lamina ascendens alæ temporalis. *lary. gloss. m.* Laryngo-glossus muscle. *lat. hyal.* Latero-hyal cartilage. *lat. m.* Lateral masticatory muscle. *lev. hy. m.* Levator hyoidei muscle. *lev. pal. m.* Levator veli palatini muscle. *ling. n.* Lingual nerve. *ling. & chorda ty.* United lingual and chorda tympani nerves. *ling. br. IX.* Lingual branch of ninth. *mal. b.* Malar bone. *mall.* Malleus portion of Meckel's cartilage. *Mam. pty. b.* Mammalian pterygoid bone. *mand.* Mandible. *mass. m.* Masseter muscle. *mass. n.* Masseter nerve. *max. b.* Maxilla bone. *Me.* Meckel's cartilage. *med. m.* Median masticatory muscle. *memb. ob.* Membrana obturatoria. *mid. cerv. v.* Middle cervical vein. *Mon. pty. b.* Monotreme pterygoid bone. *mylohy. n.* Mylo-hyoid nerve. *otic. g.* Otic ganglion. *orb. par. com.* Orbito-parietal commissure. *pal. b.* Palate bone. *pal. ns.* Palatine nerves. *par. b.* Parietal bone. *platys. m.* Platysma muscle. *phar.* Pharynx. *post. dig. m.* Posterior digastric muscle. *post. dig. m. d. p.* Posterior digastric muscle, dorsal part. *post. dig. m. v. p.* Posterior digastric muscle, ventral part. *presph. c.* Presphenoid cartilage. *premand. s.* Premandibular segment. *proc. alaris.* Process alaris. *pty. bo.* Pterygoid bone. *r. lat. V_2.* Ramus lateralis of the mandibular division of fifth. *r. med. V_2.* Ramus medialis of the mandibular division of fifth. *sph. prof.* Sphincter profundus. *sph. sup.* Sphincter superficialis. *sphen. pal. g.* Spheno-palatine ganglion. *sq. b.* Squamous bone. *stap. m.* Stapedius muscle. *sternogloss. m.* Sterno-glossus muscle. *sternohy. m.* Sterno-hyoid muscle. *st. mand. m.* Sterno-mandibularis muscle. *st. mast. m.* Sterno-mastoid muscle. *stylogloss. m.* Stylo-glossus muscle. *stylhyal.* Stylo-hyal cartilage. *stylph. m.* Stylo-pharyngeus muscle. *styloid. m.* Styloideus muscle s. hyoid ventral constrictor. *sub. max. gl.* Submaxillary gland. *temp. m.* Temporal muscle. *tend. post. dig.* Tendon of posterior digastric muscle. *tend. tens. pal.* Tendon of tensor veli palatini muscle. *tens. pal. m.* Tensor veli palatini muscle. *tens. ty. m.* Tensor tympani muscle. *thyrohy. m.* Thyro-hyoid muscle. *thyro. cart.* Thyroid cartilage. *trap. m.* Trapezius muscle. *tymp. b.* Tympanic bone. *vid. n.* Vidian nerve.

zygo. mand. m. Zygomatico-mandibularis muscle. Roman numerals. Cranial nerves.

Dasyurus viverrimus, figs. 1–28.

Figs. 1–3.—From transverse sections, stage A (just born; greatest length, in spirit, 5·5 mm., head length 2·5 mm.); fig. 1 is the most anterior. Fig. 1, slide 1, row 1, number 17; fig. 2, s. 1, r. 7, n. 3; fig. 3, s. 1, r. 8, n. 12.

Fig. 4.—From transverse section, stage B (few hours old; greatest length 5·75 to 6 mm. head length 3 mm.) : the section is a little oblique and the right side is anterior to the left ; s. 2, r. 1, n. 1.

Fig. 5.—Portion of transverse section, stage B ; s. 2, r. 1, n. 20, showing undifferentiated mesoderm between posterior digastric muscle and epiblast.

Figs. 6–10.—From sagittal sections stage C (twenty-six hours old; greatest length 6 mm. head length 3·25 mm.); fig. 6 is the most anterior. Fig. 6, s. 1, r. 2, n. 1; fig. 7, s. 1, r. 3, n. 6; fig. 8, s. 1, r. 4, n. 1; fig. 9, s. 1, r. 4, n. 7; fig. 5, s. 1, r. 5, n. 9.

Fig. 11.—From transverse section stage C ; s. 2, r. 2, n. 15.

Fig. 12.—Portion of transverse section, stage C, s. 2, r. 3, n. 8, showing stapedius muscle.

Fig. 13.—Portion of transverse section, stage C, s. 2, r. 4, n. 11, showing Anlage of sphincter profundus and platysma muscle.

Figs. 14–18.—From transverse sections, stage D (probably three days old ; greatest length 7 mm., head length 4 mm.); fig. 14 is the most anterior. Fig. 14, s. 1, r. 8, n. 1; fig. 15, s. 1, r. 8, n. 9; fig. 16, s. 1, r. 8, n. 13; fig. 17, s. 2, r. 1, n. 3; fig. 18, s. 2, r. 2, n. 4.

Fig. 19.—Portion of transverse section, stage D, s. 2, r. 3, n. 12, showing stapedius muscle.

Figs. 20, 21.—From transverse sections, stage E (5 or 6 days old; greatest length 8 mm., head length 4·5 mm.); fig. 20 is the more anterior; Fig. 20, s. 2, r. 4, n. 12; fig. 21, s. 2, r. 5, n. 5.

Figs. 22, 23.—From transverse sections, stage F (about 7 days old; greatest length 8·5 to 9 mm., head length 5 to 5·5 mm.) ; fig. 22 is the more anterior. Fig. 22, s. 2, r. 8, n. 10; fig. 23, s. 3, r. 2, n. 13.

Figs. 24–27.—From transverse sections, stage H (about 14 days old; greatest length 13·5 mm., head length 8 to 8·5 mm.) ; fig. 24, is the most anterior. Fig. 24, s. 5, r. 3, n. 10; fig. 15, s. 5, r. 5, n. 2; fig. 26, s. 5, r. 6, n. 8; fig. 27, s. 6, r. 2, n. 10.

Fig. 28.—From transverse section, stage J (25 days old; greatest length 20 mm., head length 12·5 mm.) ; s. 3, r. 2, n. 5.

Ornithorhynchus, figs. 29–33.

Figs. 29–31.—From longitudinal sections. embryo 8·5 mm.; fig. 29 is the most lateral. Fig. 29, s. 3, r. 2, n. 4; fig. 30, s. 4, r. 1, n. 3; fig. 31, s. 4. r. 3. n. 4.

Figs. 32, 33.—From transverse sections, embryo 8·5 mm. ; fig. 32 is the more anterior. Fig. 32, s. 8. r. 1. n. 12 ; fig. 33, s. 7, r. 1, n. 3.

Echidna. figs. 34–38.

Figs. 34–37.—From transverse sections, 25 mm. long (= stage 50 of Semon) ; fig. 34 is the most anterior.

Fig. 38.—From transverse section, young adult 12·5 cm. long.

Fig. 39.—Transverse section. Didelphys aurita, 10 mm.

Fig. 40.—Sketch copied from Bijvoet, showing digastric muscle of Bradypus tridactylus.

Fig. 41.—Sketch showing digastric muscle of Bradypus marmoratus.

Fig. 42.—From transverse section. Dasypus novemcincta, 30 mm.

Fig. 43.—Sketch of side of head of Manis pentadactyla.

Fig. 44.—Sketch of muscles on inside of jaw of Phoca vitulina.

Rabbit. figs. 45–54.

Fig. 45.—Transverse section. embryo 5¼ mm. crown-rump length.

Figs. 46–48.—Transverse sections. embryo 16 mm. crown-rump length; fig. 49 is the most anterior.

Figs. 49–53.—Longitudinal horizontal sections, embryo 23 mm. crown-rump length ; fig. 54 is the most dorsal.

Fig. 54.—Longitudinal horizontal section, embryo 33 mm. crown-rump length.

Pig. figs. 55–64.

Figs. 55–58.—Transverse sections embryo 15 mm. crown-rump length; fig. 60 is the most anterior.

Fig. 59–63.—Transverse sections, embryo 21 mm. crown-rump length; fig. 68 is the most anterior.

Fig. 64.—Transverse section. embryo 32 mm. crown-rump length.

Text-figures.

Figs. 65–67.—Sketches of model of developing masticatory muscles of rabbit, embryo 13 mm. crown-rump length ; fig. 65 from inside, fig. 66 from front, fig. 67 from outside.

Figs. 68, 69.—Sketches of model of developing masticatory muscles of rabbit, embryo 16 mm. crown-rump length ; fig. 68 from inside, fig. 69 from outside.

Figs. 70, 71.—Sketches of model of developing masticatory muscles of pig, embryo 19 mm. crown-rump length ; fig. 70 from inside, fig. 71 from outside.

Figs. 72, 73.—Sketches of model of developing masticatory muscles of pig, embryo 21 mm. crown-rump length ; fig. 72 from inside, fig. 73 from outside.

Figs. 1–28, 39–41, 43 were drawn by Mr. E. E. Shellard.

Figs. 65–73 were drawn by Mr. C. W. Sharpe.

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TAYLOR — SYMBRANCHUS.

ROBERTSON — HEART OF LEPIDOSIREN.

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10.

MARTIN ON TRYPANOPLASMA.

MARTIN ON TRYPANOPLASMA.

HASWELL — STRATIODRILUS.

HASWELL — STRATIODRILUS

HASWELL — STRATIODRILUS.

HASWELL — STRATIODRILUS.

DIAGRAM 4.

DIAGRAM 6.

DIAGRAM 5.

GOODRICH — METAMERIC SEGMENTATION.

GOODRICH — METAMERIC SEGMENTATION.

MEEK— SPERMATOGENETIC MITOSIS.
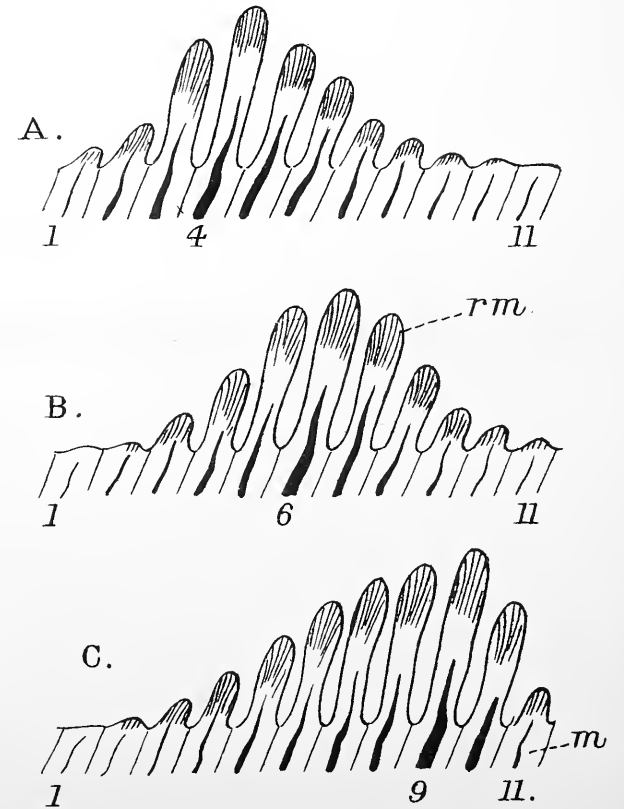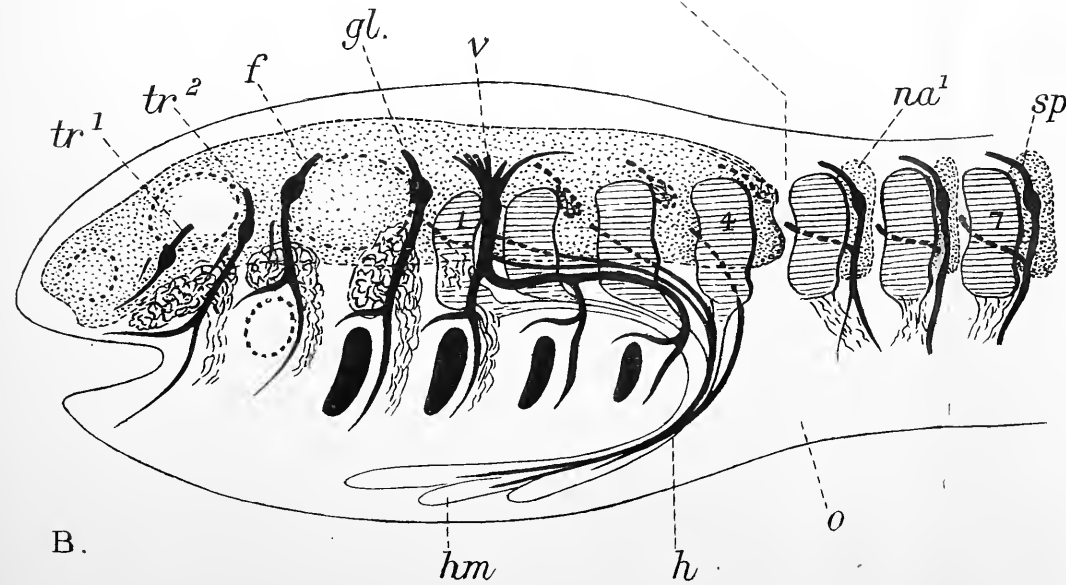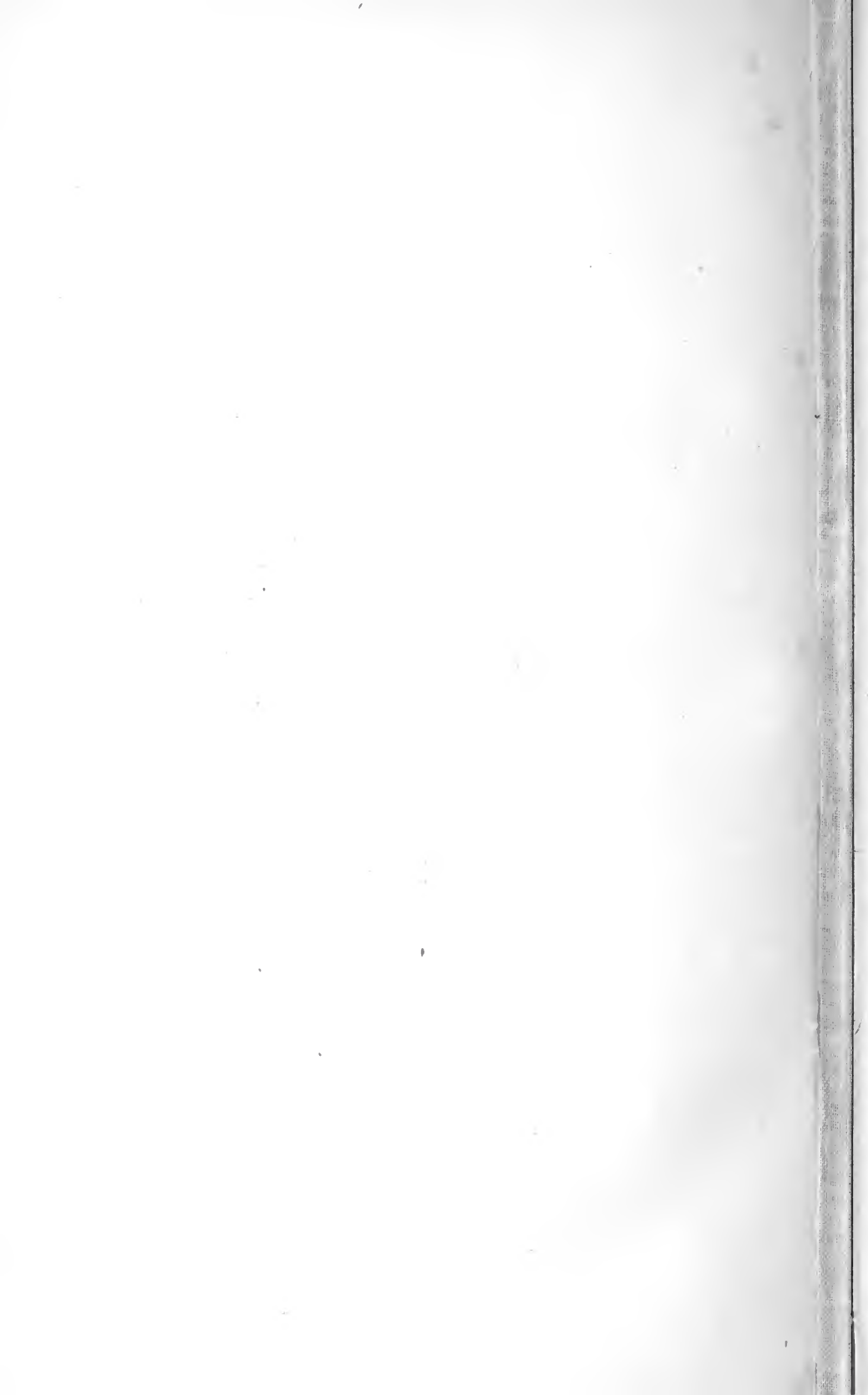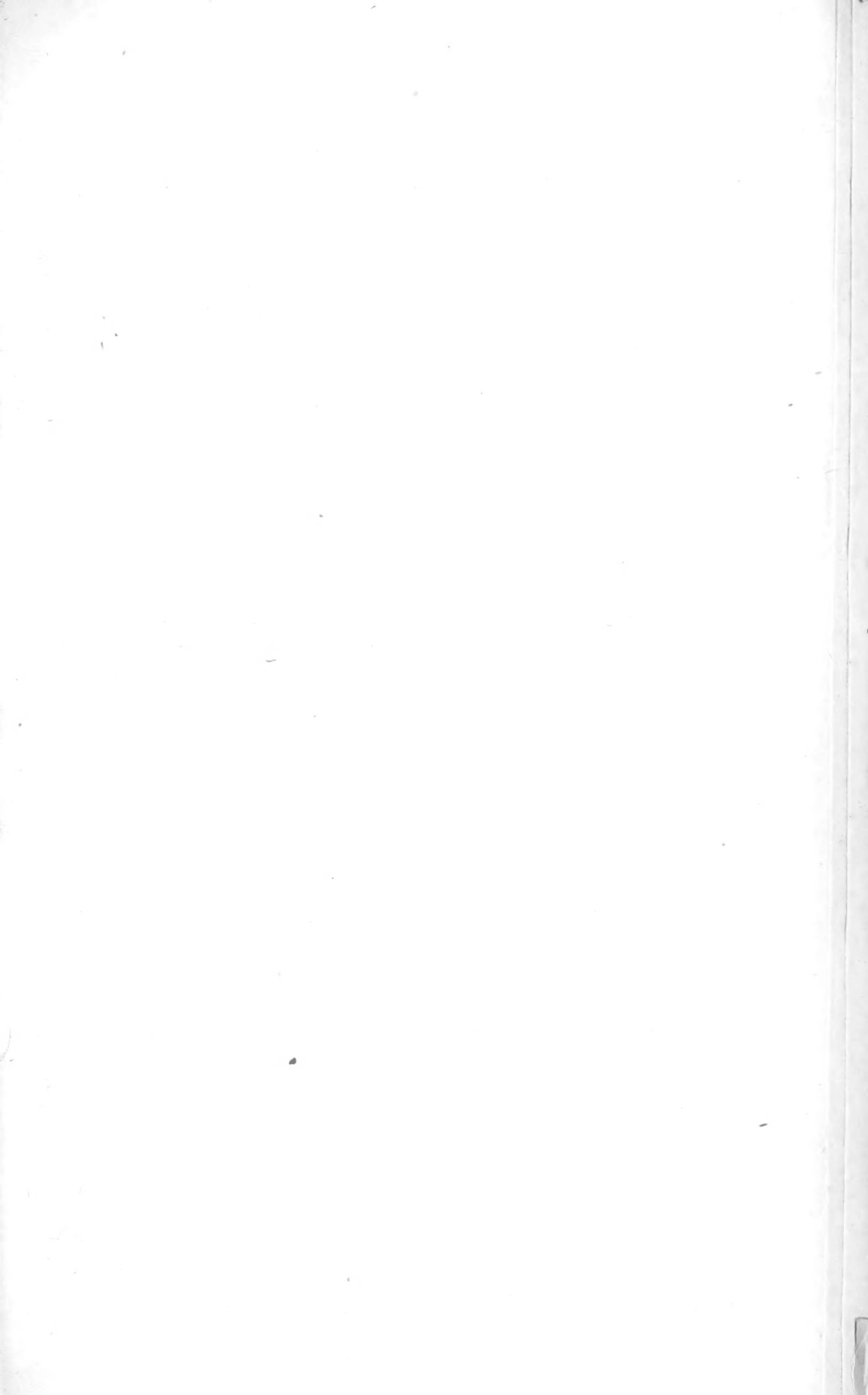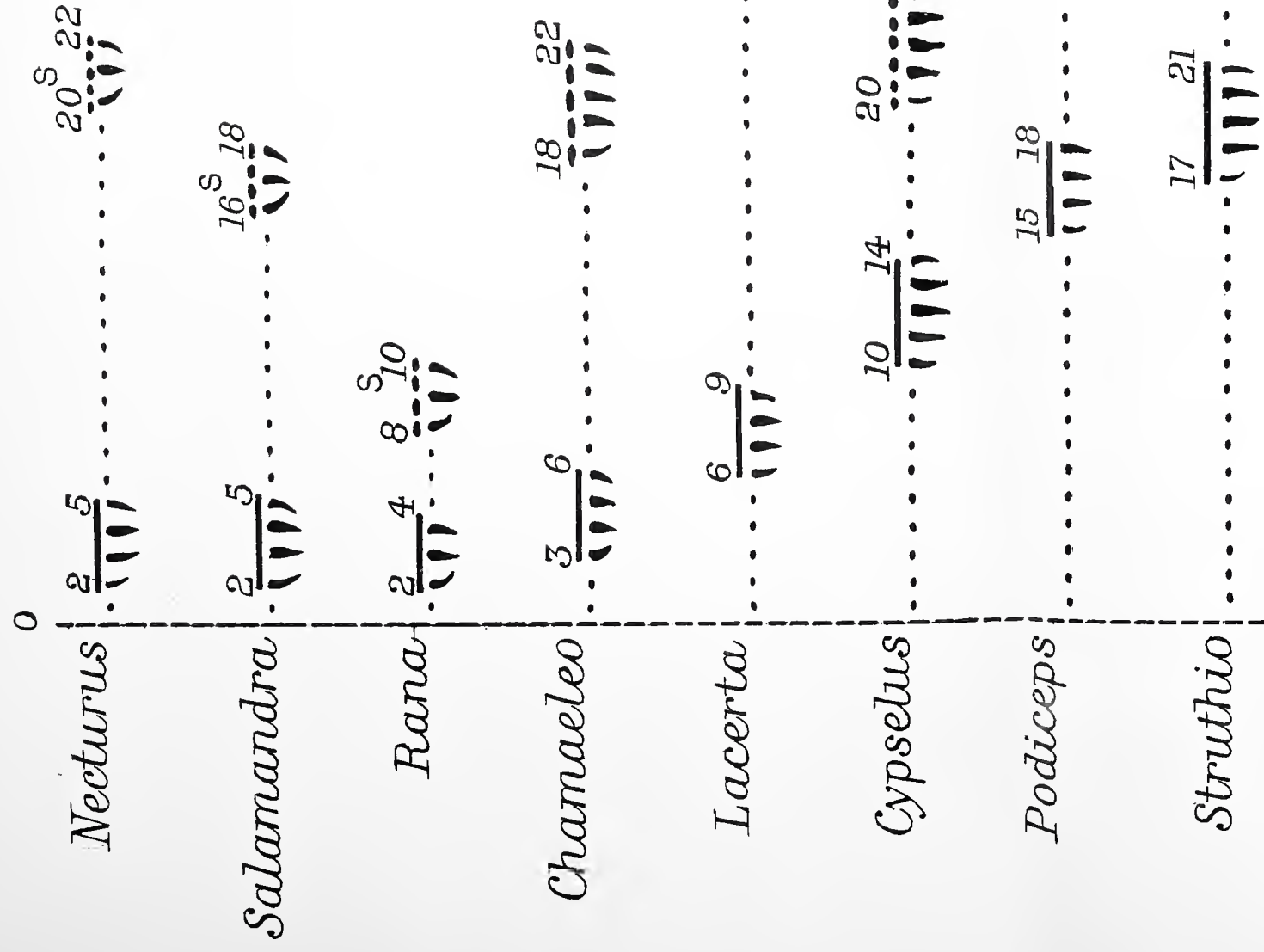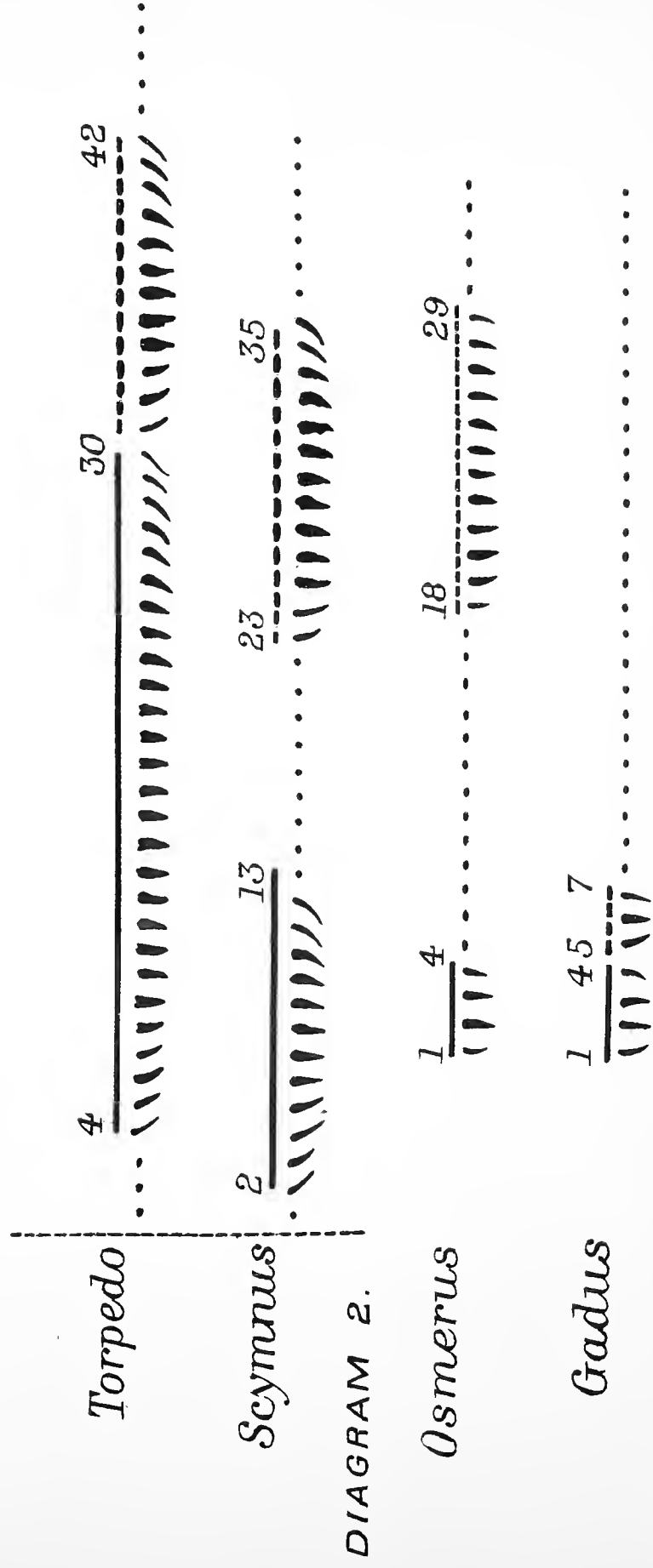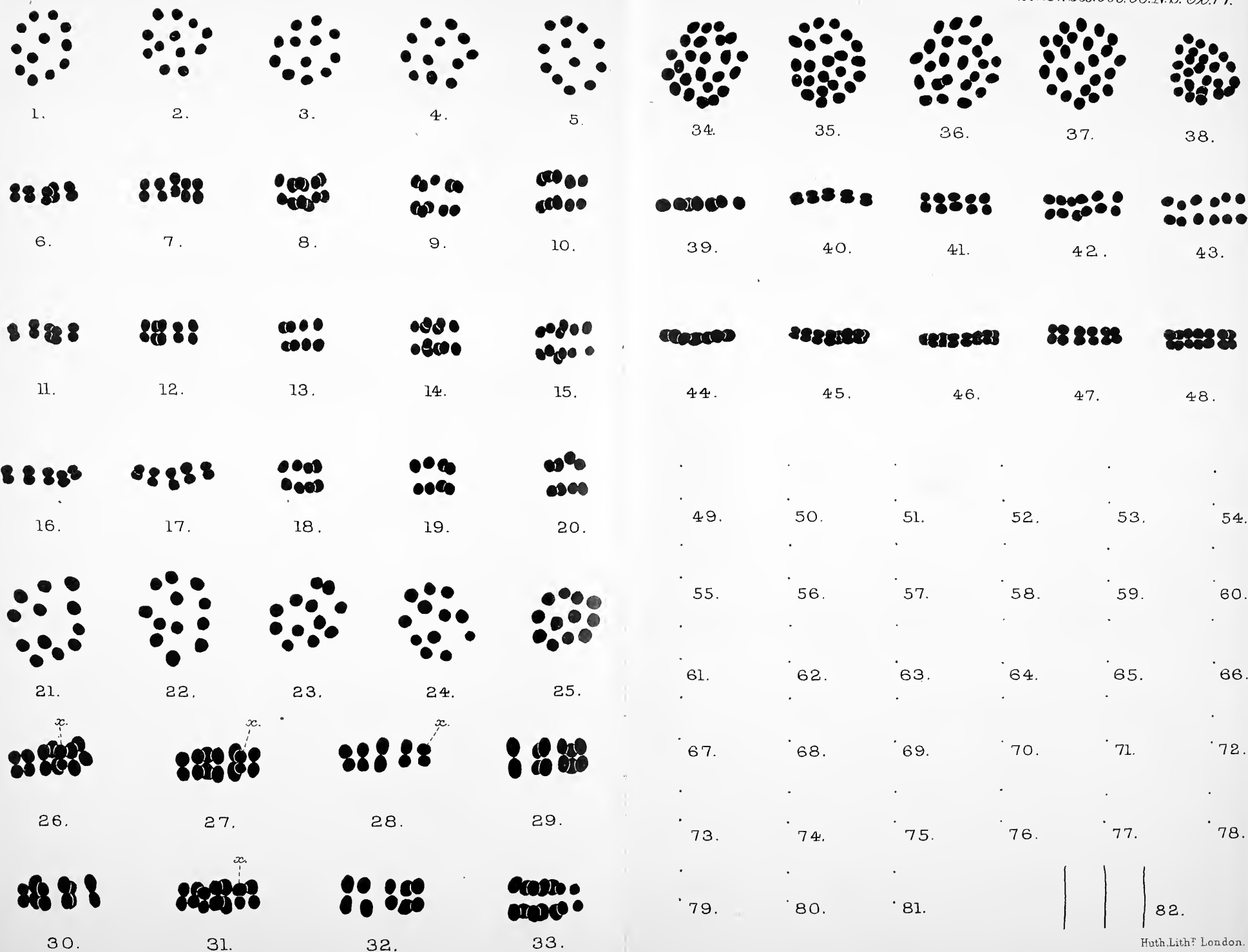
MACKINNON ON POLYMASTIX.

LYMPHATICS OF POLISTOTREMA, FIGS 1–5 AND 7.

ADLARD & SON IMP'R

ADLARD & SON, IMPR.

LYMPHATICS OF POLISTOTREMA, FIGS. 10 AND 13—25.

JOHNSTON TREMATODES.

JOHNSTON — TREMATODES.

JOHNSTON — TREMATODES.

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WOODCOCK and LAPAGE — PROTISTAN PARASITE.

WOODCOCK AND LAPAGE — PROTISTAN PARASITE.

Fig. 1.

memb. ob, lat. m., ala. temp, inf. dent. n., mand, Me., gen. hy. m., intermand. m., med. m., r. med. V₃, orb. par. com, r. lat. V₃

Fig. 2.

ala. temp, orb. par. com., lat. m., Gass. g., V₃, lat. m, med. m., inf. dent. n., ling. & chorda ty, Me., intermand. m., XII., gen. hy. m.

Fig. 3.

stap. m., VII., Eust. tb., intermand. m., stylhyal, stylhyal, hyogloss. m., gen. hy. m., st. phar. m., psot. dig. m.

Fig. 7.

VI., II., Pty. int. m., An. of tens. pal. & tens. tymp., VII., pal. b., stap. m., stylhyal, Eust. tb., An. of sph. prof. & platys. m.s, mand., Me., mylohy. n., chorda. ty.

Fig. 6.

E., V₂, orb. par. com., gass. g., incus, E.A.M., mass. m., bucc. n., lat. m., aur. temp. n., Me.

Fig. 4.

Eust. tb., or., incus., med. m., stap. m., VII., styl. hyal., styl. gloss. m., post. dig. m., gen. hy. m., XII., hyogloss. m., styl. gloss. m., intermand. m., Me.

Fig. 9.

pty. b., ala. temp., An. of tens. pal. & tens. temp., pal. b., IX., styl. hyal., Me., gen. gloss. m., stylngl. m., post. dig. m., submax. gl., intermand. m.

Fig. 8.

ala. temp, II., pal. b., pal. b., Eust. tb, post. dig. m., styl. hyal., An. of sph. prof. & platys., int. ply. m., intermand. m., Me.

Fig. 5.

post. dig. m., Dorsal, Ventral

Fig. 10.

Fig. 11.

Fig. 12.

Fig. 13.

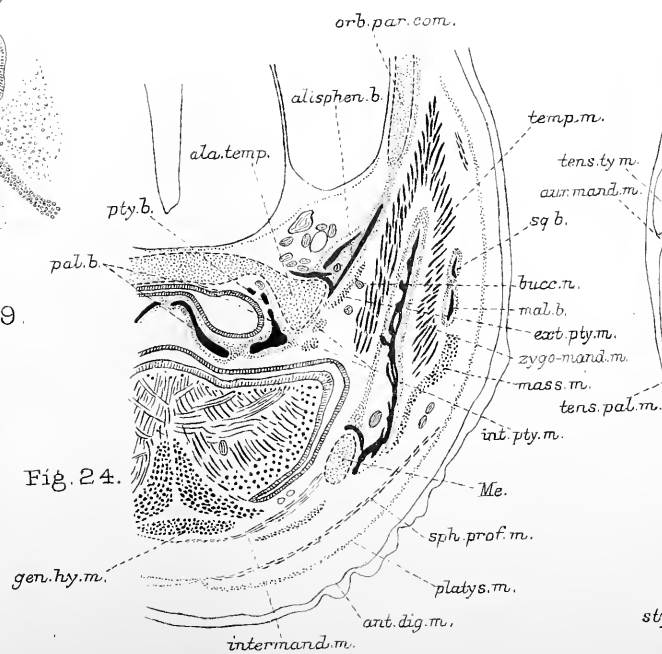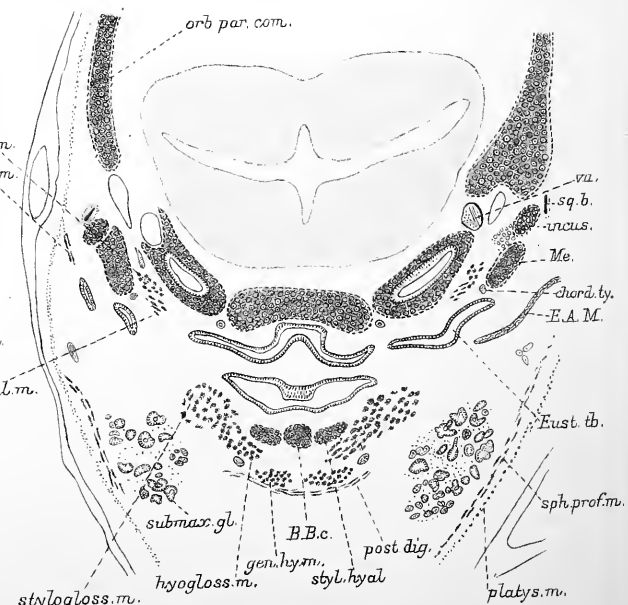Fig. 14.

Fig. 15.

Fig. 16.

Fig. 17.

Huth, Lith. London.

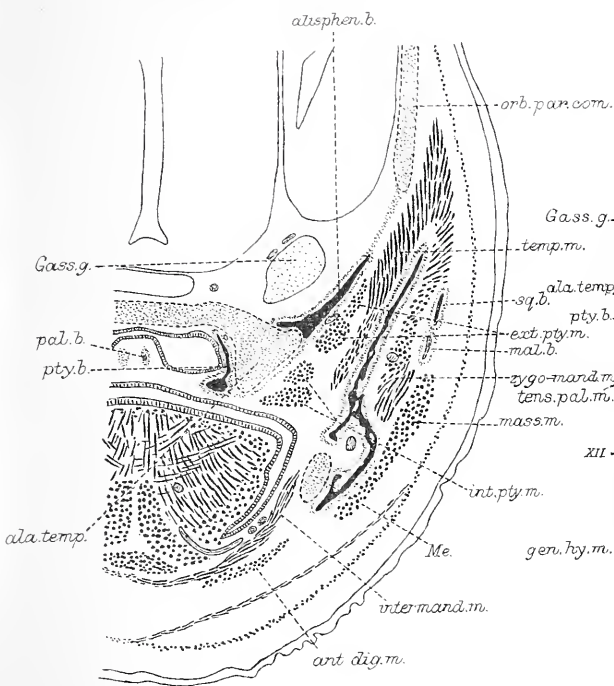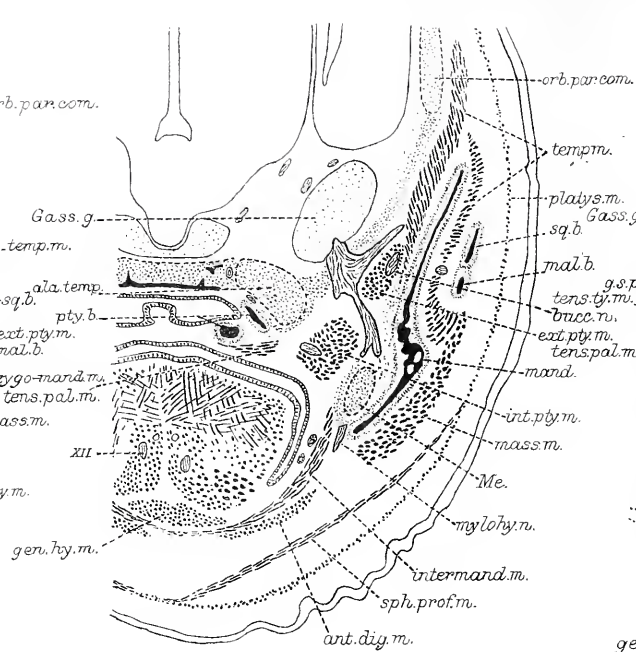Fig. 18.

Fig. 19.

Fig. 20.

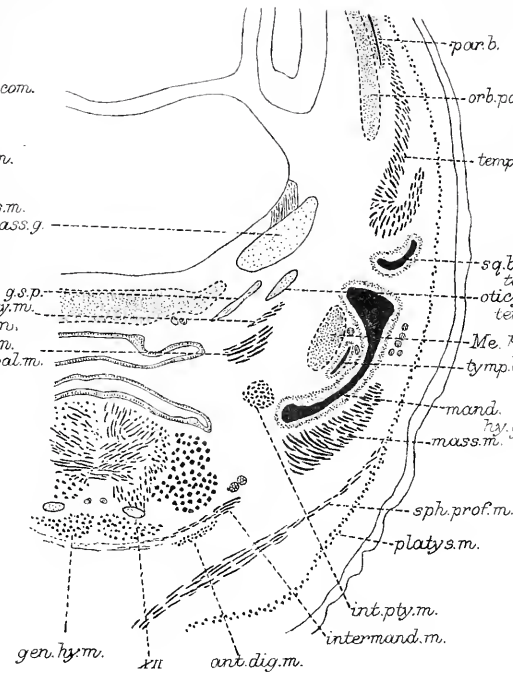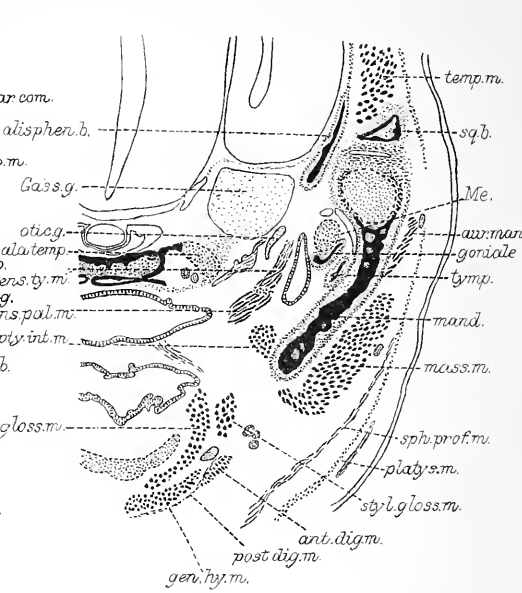Fig. 21.

Fig. 22.

Fig. 23.

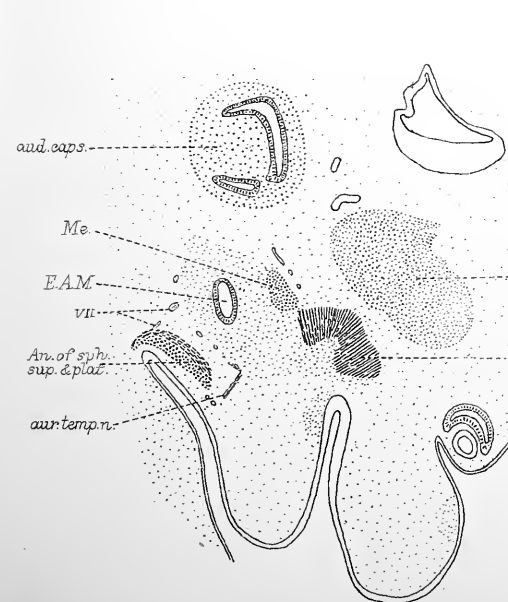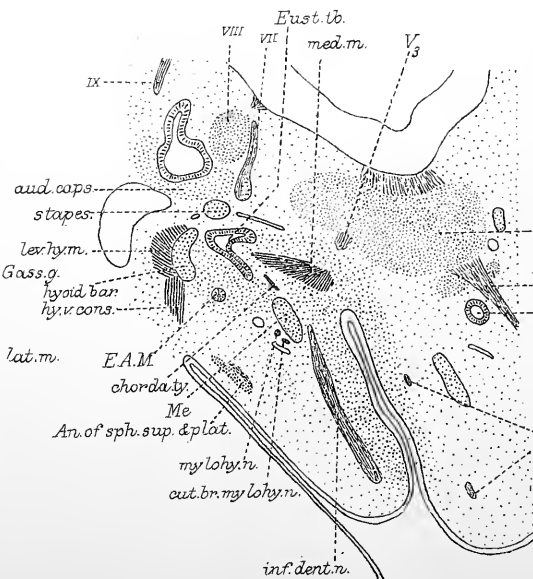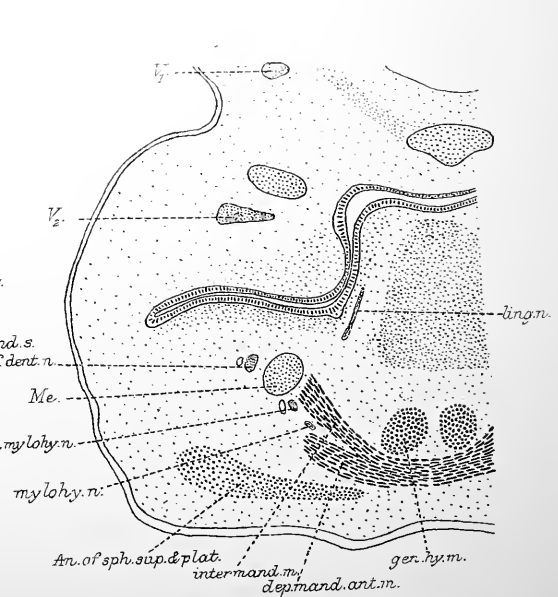Fig. 24.

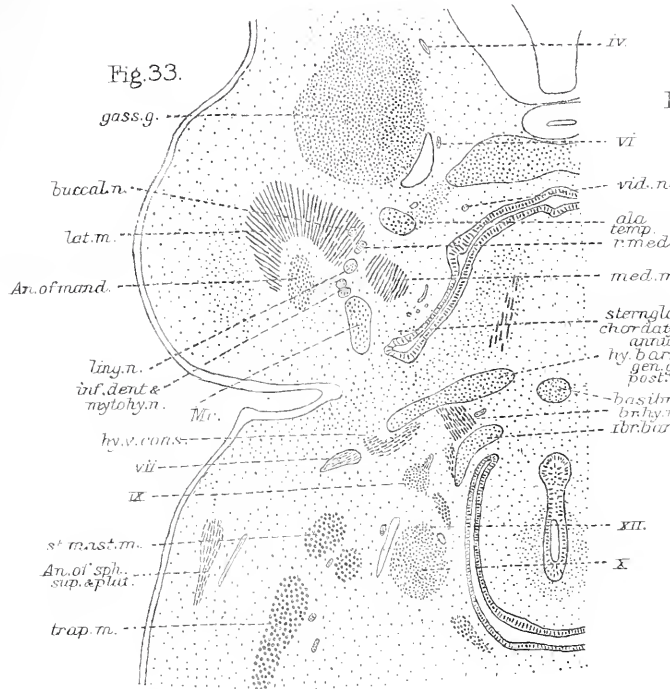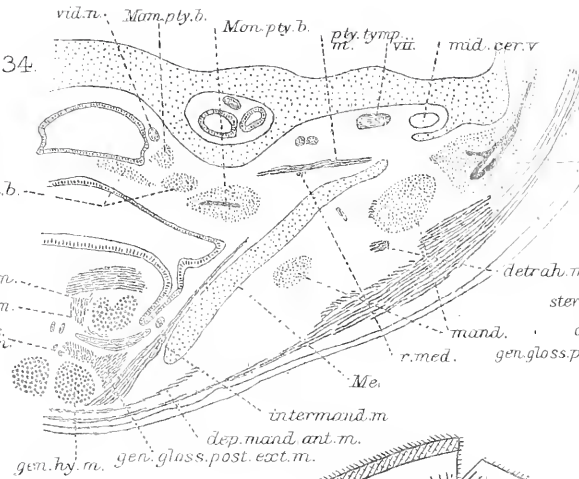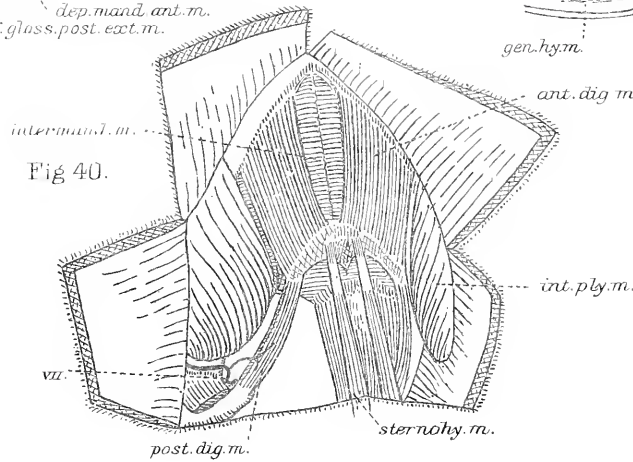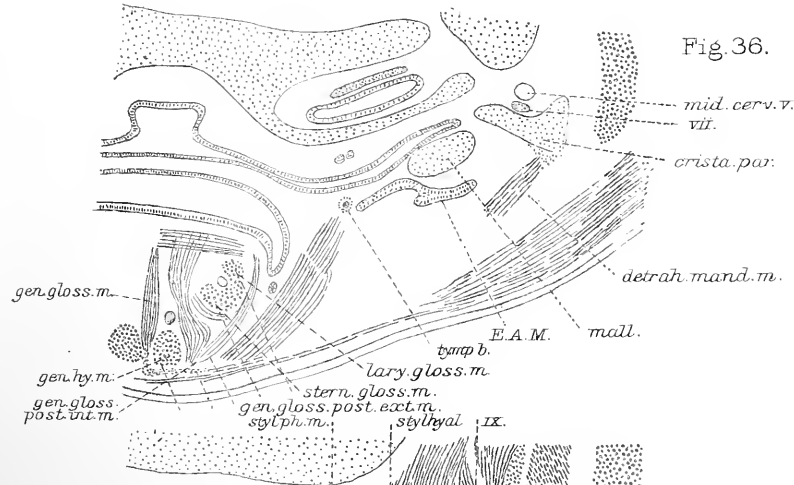Huth, Lith. London.

Fig. 25.

Fig. 26.

Fig. 27.

Fig. 28.

Fig. 29.

Fig. 30.

Fig. 31.

Fig. 32.

Fig. 33.

gass.g.

buccal.n.

lat.m.

An.of mand.

ling.n.
inf.dent.
mylohy.n.
Mc.

hy.v.cons.

vii

ix

st.mast.m.
An.of sph.
sup.&prof.

trap.m.

IV.

VI

vid.n.

ala
temp. *pal.b.*
r.med. V3

med.m.

sterngloss.m.
chordaty.n.
annulus m.
hy.bar.
gen.gloss.
post.int.m.
basibr.o.
br.hy.m.
tbr.bar.

XII.

X

Fig. 34.

vid.n. *Mon.pty.b.* *Mon.pty.b.* *pty.tymp.*
mt. *vii* *mid.cerv.v*

sq.b.

detrah.mand.m.

sterngloss.m.
annulus.m.
mand. *gen.gloss.post.int.m.*
r.med.

Me.

intermand.m.
dep.mand.ant.m.
gen.hy.m. *gen.gloss.post.ext.m.*

Me. *incus.* *vii.* *mid.cerv.v.*

sq.b.

mand.

detrah.mand.m.

tens.ty.m.

Mon.pty.b.
intermand.m.
dep.mand.
ant.m.
gen.hy.m. *gen.gloss.post.ext.m.*

Fig. 35.

Fig. 40.

intermand.m.

vii.

post.dig.m.

ant.dig.m.

int.ply.m.

sternohy.m.

Fig. 41.

mass.m.

ant.dig.m.

int.ply.m.

vii.

post.dig.m.d.p.
stylhyal

stylhyal
sternohy.m.
post.dig.m.v.p.

Fig. 36.

mid.cerv.v.
vii.

crista.par.

detrah.mand.m.

gen.gloss.m.

gen.hy.m.
gen.gloss
post.int.m.

E.A.M.
tymp.b.
lary.gloss.m.
stern.gloss.m.
gen.gloss.post.ext.m.
stylph.m. *stylhyal* *IX.*

mall.

Fig. 38.

vii.

st.mast.m.

vii.
lev.hy.m.

hy.v.cons.

crista.par

stapes

Eust.th.

sb.hyal

Fig. 37.

mid.cerv.v.
vii.

gen.gloss.m.

E.A.M.
hy.v.cons.m.
intermand.m.
lary.gloss.m.
sternogloss.m.
gen.gloss.post.ext.m.
gen.hy.m. *gen.gloss.post.int.m.*

lev.hy.m.

Fig. 39.

sq.b.

incus

aur.mand.m.
tens.ty.m.
tymp.b.
tens.pal.m.

E.A.M.

tymp.b.

mand.
mass.m.

int.ply.m.
sph.prof.m.
platys.m.

intermand.m.

gen.gloss.m.
gen.hy.m.

int.ply.m.
mass.m.

mand.

Me.

ant.dig.m.

Fig. 42.

Fig. 43.

Fig. 46.

Fig. 47.

Fig. 49.

Fig. 44

Fig. 48.

Fig. 45.

Fig. 50.

Fig. 51.

pal. n.s.
mass. & zyg. mand.
An. of pal. & pty. b.s
ham.
ala. temp.
otic. g.
tens. ty. m.
vii.

stapes.
mass. & zyg. mand
Me.
temp. m.
mand.
An. of hy. glos. & stylogloss
ext. pty. m.
int. pty. inf.
d. mylohyns
aur. temp. n.
ma.
mall.
incus.

Fig. 55

viii.
vii.
g. s. p.
An. of pal. & pty. b.s
int. pty. m.
chord. ty.
xii.

intermand. m.   gen. hy. m.   ant. dig. m.

mass. & zyg. mand.
temp. m.
platys. m.
mand.
Me.
aur. temp. n.
E. A. M.
mall.
incus.
stapes.
stap. m.
vii.

Fig. 53.

Fig. 52.

buccal n.
An. of pal. & pty. b.s
int. pty. m.
ham.
tend. of tens. pal.
tens. ty. m.

mal. b.
mass. & zyg. mand.
mand.
temp. m.
platys. m.
inf. d. ling. & mylohy. n.
chorda ty. n.
aur. temp. n.
Me.
mall.
incus
vii.

Fig. 57.

br. of vii to g. sp.
vii.
br. of ix to g. s. p.
styl. hyal.
vii.
An. of sph. prof. & platys
stern. hy. m.

ix.
aud. ves.
An. of stap. & hy. v. cons.
ant. dig. m.
xii.

Fig. 54

max. b.
mass. m.
mal. b.
platys. m.
mass. n.
zyg. mand. m.
temp. m.
mand.
ext. pty. m.
s. q. b.
parb.
orb. par. c.

pal. b.
lam. asc. a. t.
gass. g.

Fig. 56.

vii.
chorda. ty.
vii.
xii.
gen. hy. m.

lat. hyal.
g. s. p.
styl. hyal.
An. of hy. gloss & stylogloss
An. of sph. prof. & platys
ant. dig. m.
An. of stap. & hy. v. cons.

Fig. 58.

IX.
and. ves.
com. br. ix to vii
br. of ix to g. s. p.
vii
ling. br. ix.
An. of stap. & hy. v. cons.
An. of sph. prof. & platys.
stern. hy. m.
xii.

Fig. 59.

ala temp.
temp. m.
ext. pty. m.
int. pty. m.
mylohy. n.
mass. & zyg. mand.
mass. & zyg. mand.
Me.
mand.
ling. n.
intermand. m.
ant. dig. m.
hyogloss. m.
gen. hy. m.
gen. gloss. m.
tens. pal. m.
platys. m.

Fig. 60.

gass. g.
g. s. p. n.
tens. pal. m.
Me.
stylgloss. m.
hyogloss. m.
intermand. m.
gen. hy. m.
XII.
ant. dig. m.
platys. m.

Fig. 62.

lat. hyal
vii
stap. m.
temp. m.
styl. hyal.
otic. g.
vii
tens. pal. m.
aur. temp. m.
hy. v. cons.
vent. p.
int. pty. m.
ant. dig. m.
mand.
sph. prof. m.
br. hy. m.
IX
XII
Me. E.A.M.
styl hyal
styl hyal
vii 1st. br. bar.
hy. v. cons.
vent. p.
ant. dig. m.
1st. br. bar.
platys. m.
ant. dig. m.
hy. v. cons. vent. p.
stern. hy. m.
XII

Fig. 61.

gass. g.
genic. g.
otic. g.
tens. ty. m.
mall.
styl. gloss. m.
hyo. gloss. m.
platys. m.
sph. prof. m.
XII

Fig. 63.

vii
incus.
stapes
chorda ty
styl. hyal.
hy. v. cons. vent. p
ant. dig. m.
1 br. bar.
platys. m.
vii
lat. hyal.
stap. m.
styl. hyal.
vii.
jug. hy. m.
post. dig. m.
IX.
XII.

Fig. 64.

lat. hyal.
aur. mand. m.
Eust. tb.
E.A.M.
styl. hyal. m.
IX
XII
ant. dig. m.
br. hy. m.
stern. hy. m.
I.B.B.
1 br. bar.
stap. m.
vii
styl. ph. m.
dep. auris. m.
hy. ep. m.
styl. hy. m.
platys. m.
sph. prof.

Date Due